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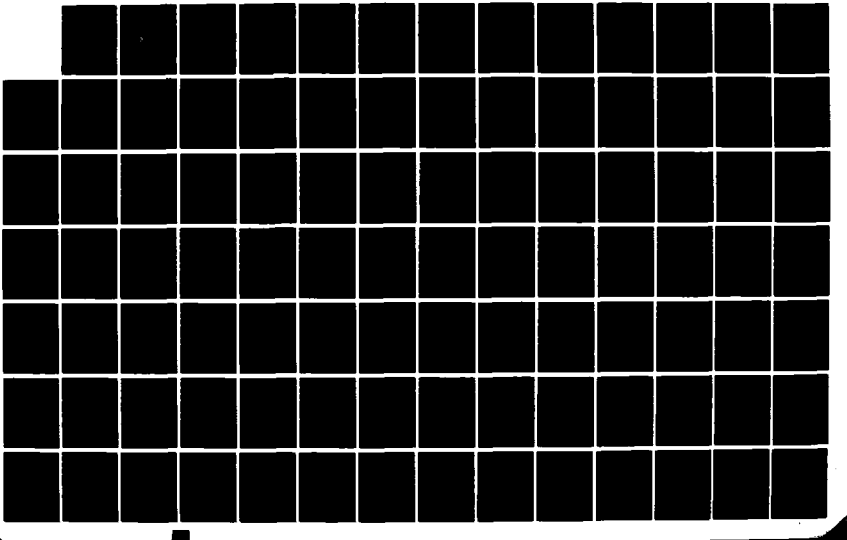

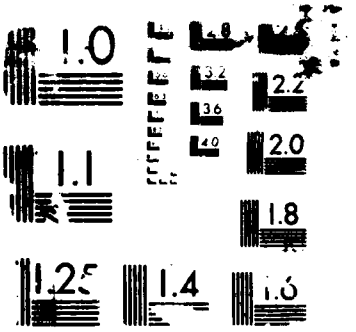
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IN VITRO TOXICITY EVALUATION OF TEN PARTICULATE MATERIALS IN TRACHEAL ORGAN CULTURE

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Columbus, OH 43201-2693

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The in vitro toxic effect of eight test particles (graphite fibers, polycrystalline iron whiskers, aluminum dust, nickel-coated graphite fibers, brass dust, Printex L carbon black, micro-260 synthetic graphite dust and KS-2 natural graphite dust) was evaluated on the upper respiratory epithelium of hamster tracheal organ cultures based on microanatomical and histomorphometric changes. The fibrous materials were ground with mortar and pestle to reduce their size to an acceptable range for the test tracheal organ culture. Tissue changes induced by the test articles were compared to changes that occurred in tracheal organ cultures exposed to crocidolite asbestos (positive control) and glass beads (negative control), in addition to untreated explants. The relative toxicity of each test particle was characterized based on qualitative histopathologic and morphometric differences relative to the positive and negative
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19. ABSTRACT (continued)

controls. A range finding study was conducted to determine relative toxicity of each test article and to select optimal test concentrations. Following range finding, explants were exposed to three concentrations of each test article and controls. The explants were examined after one and three weeks of exposure. Within the limitations of the test procedures, results showed a relative rank ordering of the potential of each test material to induce morphologically apparent pre-neoplastic lesions in tracheal organ cultures. Crocidolite asbestos and Ni-coated graphite were the most reactive in this study. Iron whiskers, aluminum dust, and KS-2 graphite caused an intermediate level of tissue response, while the remaining substances did not cause significant tissue responses compared to control explants. The most cytotoxic materials were brass dust and Ni-coated graphite.

PREFACE

This study was conducted to explore alternative toxicological testing methods for assessing the potential health hazard of particulate materials. As noted in the discussion section of the report and in the following paragraphs, there are many limitations on this in vitro testing model. The results of this study can not be interpreted as an indicator of absolute toxicity. However, as a preliminary evaluation, a relative toxicity ranking can be assigned to the test materials. The ultimate determinant of airborne hazard still lies within the purview of standard inhalation protocols. In vitro testing procedures may reduce the number of animals required to screen chemicals but in vivo inhalation testing can not be replaced at this time.

Scientists at the U.S. Army Chemical Research, Development and Engineering Center (CRDEC) questioned why some of the diameters are so large and indicated that their measured mass median aerodynamics diameters (MMAD) of several of these dusts were smaller. One possible explanation for this is that our data reflect measured count median diameter (CMD) from which the mass median diameter (MMD) was calculated, whereas we presume CRDEC data were derived from an aerosol of each material. The relationship between CMD and MMD depends upon the geometric standard deviation (GSD) of the distribution of particle diameters.

$$\ln MMD = \ln CMD + 3 \ln^2 GSD$$

If GSD = 1, which is true for mono dispersed aerosols (i.e., all particles are the same size), then the MMD = CMD. In all other cases, the GSD is greater than 1, and the MMD will be greater than CMD.

For example, if CMD = 2 μm and GSD is 1.5, 2, or 2.5 the MMD will be 3.28 μm, 8.45 μm, or 24.83 μm, respectively. These are dramatic differences in mass medium diameter associated with small changes in the GSD.

The relationship between real diameter, d_p, and aerodynamic equivalent diameter, d_{ae}, depends upon the density, δp, and the slip correction, C, for the size, d.

$$\sqrt{C_{ae}\ \delta_{ae}}\ d_{ae} = \sqrt{C_p\ \delta_p}\ d_p$$

Because $P_{ae} = 1$ and C_{ae} depends upon d_{ae}, the aerodynamic equivalent diameter can only be determined by successive approximations. Frequently, the product $\sqrt{C_{ae}\ d_{ae}}$ is used as the effective aerodynamic equivalent diameter. Values of C_p vary from 2.87 at 0.1 μm to 1.16 at 1 μm to 1.02 at 10 μm, so the conversion from real size to aerodynamic equivalent size is not a simple extrapolation. When aerodynamic equivalent size distribution parameters are quoted, they were usually obtained with equipment that was calibrated using unit density spheres.

In addition, because we do not know how CRDEC generated the dusts to measure MMAD, which can have a profound effect on the particle size distribution of the resultant aerosol, it is difficult for us to compare the two data sets any further.

CRDEC scientists' also asked why the asbestos is less cytotoxic than the media control at 3 weeks, referring to Table 13, which listed total percent of tracheal explants bearing mucosal lesions. We made a clarification in this table, indicating that explants often showed more than one lesion and, therefore, the total value does not add up to 100%. The observation of the different incidences of cytotoxity at 3 weeks is because most of the asbestos-exposed tracheas developed proliferative or metaplastic lesions, which either replaced or obscured degenerative changes. Additionally, the cytotoxic changes included for media control tissues simply reflect a large number of explants that had minimal cuboidal to squamous change, which as explained in the report, is a common finding in long-term tracheal explants and does not represent toxic injury.

The work described in this report was authorized under Contract No. DAAK11-82-D-0008. This work was started in June 1985 and completed in June 1987.

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animal Resources, National Research Council.

This report has been approved for release to the public.

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IN VITRO TOXICITY EVALUATION OF TEN PARTICULATE MATERIALS IN TRACHEAL ORGAN CULTURE

from

BATTELLE
Columbus Division

1. INTRODUCTION

The tracheal organ culture model has been shown to be an effective short-term in vitro assay for the detection of cytotoxic and genotoxic damage that is induced by a variety of toxic compounds[2,7]. Mucosal epithelium of tracheal explants has been reported to develop putative pre-neoplastic lesions following exposure to a spectrum of carcinogens, including particulate materials having carcinogenic properties. These in vitro lesions share similar histopathologic features with pre-neoplastic changes observed in animals and humans following exposure to carcinogenic particles[2,3,5,6,7]. For example, in vitro exposure to asbestos causes squamous metaplasia and mucosal hyperplasia with frequent dysplastic features in tracheal epithelium[2,3] whereas, tracheal explants exposed to inert particles (e.g., glass beads, micronized latex particles, or nuisance dusts) have a much lower incidence of histological changes and changes that do occur, do so after exposure to higher comparative concentrations, are generally cytotoxic in nature and of mild severity. In this study the in vitro toxic effect of eight test particles (graphite fibers, polycrystalline iron whiskers, aluminum dust, nickel-coated

graphite fibers, brass dust, Printex L carbon black, micro-260 synthetic graphite and KS-2 natural graphite) on the upper respiratory epithelium of hamster tracheal organ cultures was evaluated based on microanatomical and histomorphometric changes. Tissue changes induced by the test articles were compared to changes that occur in tracheal organ cultures exposed to crocidolite asbestos (positive control) and glass beads (negative control), in addition to untreated explants. The relative toxicity of each test particle was characterized based on qualitative histopathologic and morphometric differences relative to the positive and negative control groups.

2. MATERIALS AND METHODS

2.1 Test Materials.

Eight particulate test materials were received at Battelle from CRDEC on June 19, 1985. These were identified as KS-2 natural graphite, micro-260 synthetic graphite, carbon black (Printex L), aluminum powder and brass powder. In addition, graphite fibers, nickel-coated graphite fibers and polycrystalline iron whiskers, were received November 22, 1985. Crocidolite asbestos and micronized glass beads (stock reference samples) were used in these studies as positive and negative control materials, respectively. The graphite fibers, Ni-coated graphite fibers and polycrystalline iron whiskers were too large in size as received, to suspend in media and expose tissues and extract any meaningful data. Therefore, each was ground with mortar and pestle into smaller particles. All of these fibers were ground in 100 percent ethanol to minimize particle lost and dust production. Ground samples were air dried overnight.

The percent silica in each test and control material was determined by inductive emission colorimetric spectroscopy. The method used for silica determination is included in Appendix A. In addition, 100 milligrams of each positive control, negative control, and test particle was sent to Particle Data Laboratories, LTD, for Electrozone Analysis or analytical image analysis to determine the particle size distribution of each sample. Brass dust,

aluminum dust, Printex L, micro-260 synthetic graphite, crystalline iron whiskers, KS-2 natural graphite, crocidolite asbestos, and the glass beads were analyzed by electrozone analysis. The particle size distribution of the ground graphite fibers and nickel coated graphite fibers were determined by image analysis. The methods for each of these analyses is included in Appendix A.

2.2 Explant System.

Female golden Syrian hamsters (ages 25-42 days old) were used as the trachea donor animals for this study. The hamster tracheal system was selected based on the large amount of data available on tracheal explants exposed to a variety of xenobiotics, specifically, asbestos fibers, nuisance dusts and other particles[1-8]. Hamsters used in both the range-finding study and definitive study were purchased from Charles River Laboratories, Inc. Animals used in the range finding study were shipped from Wilmington, Massachusetts, while the hamsters used in the definitive study were shipped from Kingston, New York. The animals were shipped in filtered crates. Upon arrival, each animal was examined and its general health assessed. Animals were group housed (5/cage) in polycarbonate cages. Cages were held in a bio-clean Hazelton Porta-Room. Animals were provided tap water in bottles with sipper tubes and certified Purina Rodent Chow® ad libitum. Air temperature within the holding room was maintained at 72°F ± 3° with 40-60 percent humidity. Water and feed levels were checked daily, and the bedding was changed twice weekly.

Serum samples from ten randomly selected hamsters were collected within 24 hours of arrival at Battelle and sent to Microbiological Associates in Bethesda, Maryland and screened for the presence of titers to Sendai virus, Pneumonia Virus of Mice and Lymphocytic Choriomeningitis. All ten samples tested were negative. Tracheas were extracted from the hamsters within 7 days of receipt.

Each hamster was anesthetized with 100 mg/kg of sodium pentabartitol diluted 1:1 with normal saline, administered by an intraperitoneal injection. Animals were placed in a supine position and secured to a rodent surgery

platform. Hair was removed from the anterior half of the ventral surface and the clipped area was disinfected with repeated applications of betadine surgical scrub. Loose hair was rinsed from the board with sterile distilled water and the ventral surface of each hamster rinsed with 70 percent ethanol. Using sterile techniques, tracheas were surgically removed by making a ventral midline incision from the sternum to the point of the mandible. The skin was retracted laterally and secured with a hemostat. The trachea was exposed and separated with blunt dissection from the esophagus and connective tissue. Each trachea was removed by severing the proximal end at the larynx and the distal end at the initial bifurcation. Each trachea was transferred to a sterile 60 mm diameter culture dish. All remaining connective or muscle tissue was trimmed and the trachea opened longitudinally along the cartilagenous discontinuity. Both serosal and lumenal surfaces were gently rinsed with a 1 percent solution of penicillin, streptomycin and fungizone (PSF) in Dulbecco's PBS.

Five tracheas were pooled in a single culture dish containing fresh 1 percent PSF. Each was bisected longitudinally and each half cut into 2-4 mm^2 explants by cutting between every second and third tracheal ring, yielding 10-12 explants per trachea. Five randomly selected explants from the culture dish were transferred serosal side down to a 35 mm culture plate. Each explant was allowed to attach to the scored surface of the culture plate and was incubated in complete Eagle's minimum essential media, MEM (Gibco Laboratories) with Earle's salts, 1.5 x the standard MEM concentration of amino acids and vitamins and further supplemented with 1.0 µg/ml insulin, 0.1 µg/ml retinyl acetate, 0.1 µg/ml hydrocortisone hemiacetate, 0.1 percent PSF and 100 µg/ml gentimicin. Incubator conditions were set at 37°C in a humidified atmosphere of 95 percent air and 5 percent CO_2.

Media was changed every other day with approximately 0.75 ml of fresh media per well. Explants were acclimated to the culture conditions for approximately 2 days prior to exposure with any test article.

The project was divided into 2 separate experiments. The first was a range-finding study with the objective of determining the relative toxicity of each test article to qualitatively characterize tissue changes associated with each material over a range of concentrations and to select optimal

concentrations of each test article for a follow-up study which would induce proliferative or metaplastic changes without producing overt cytotoxicity. The second experiment was labeled the definitive study with the principle goal of histomorphometrically quantitating lesion development and rank ordering each test material as to its relative toxicity in the tracheal model.

For the range-finding study, each of the eight test articles (graphite fibers, polycrystalline iron whiskers, aluminum dust, nickel-coated graphite fibers, brass dust, Printex L carbon black, micro-260 synthetic graphite and KS-2 natural graphite) and two control particles (crocidolite asbestos--positive control, and glass beads--negative control) were suspended in culture media at 6 different concentrations by serial dilution. Twenty explants were exposed to each concentration of each particulate suspension and one-half of the organ cultures (10 explants) were collected one week after exposure, while the second half of each group was collected 3 weeks after exposure. Twenty unexposed explants (media controls) per collection time were included for additional comparisons. The dose regimen for the range-finding study was as follows:

Group	Concentration	Number of Explants
1	1 µg/ml	20
2	10 µg/ml	20
3	100 µg/ml	20
4	1 mg/ml	20
5	10 mg/ml	20
6	100 mg/ml	20
7	media alone	40

The experimental design for the definitive study was similar; exposing 30 explants each, to 3 concentrations of each test article, with 60 unexposed explants serving as media controls. One-half of each group was collected one week after exposure, while the remaining tissues were collected 3 weeks after exposure. The dose regime for the definitive study was as shown below.

Glass beads, crocidolite, polycrystalline iron whiskers, graphite fibers, aluminum dust, KS-2 natural graphite, micro-260 graphite and Printex L carbon black were delivered at the following concentrations:

Group	Concentration	Number of Explants
1	100 µg/ml	30
2	1 mg/ml	30
3	10 mg/ml	30

Nickel-coated graphite fibers were formulated at concentrations of:

Group	Concentration	Number of Explants
1	10 µg/ml	30
2	100 µg/ml	30
3	1 mg/ml	30

Brass dust was formulated at concentrations of:

Group	Concentration	Number of Explants
1	1 µg/ml	30
2	10 µg/ml	30
3	100 µg/ml	30
4	1 mg/ml	30

An additional dose group (1 µg/ml) was included for the brass dust due to marked cytotoxicity observed in the range-finding study to ensure survival of some explants exposed to brass dust.

Suspensions in media of each test material were pipetted onto the lumenal surface of explants. Following a 2 hour exposure period, the particle laden media was removed and fresh media added. Tissues were collected at either 1 or 3 weeks post exposure in 10 percent neutral buffered formalin, embedded in paraffin sectioned at 3-5 µm and stained with hematoxylin and eosin. All tracheal organ cultures collected from both studies were examined by light microscopy and mucosal changes were described and graded according to distribution and severity. In the range-finding study, particular attention was given to metaplastic, dysplastic, proliferative or undifferentiated lesions, dose-response relationships associated with these lesions and the cytotoxic potential of each material. Doses for the definitive study were selected in an attempt to avoid excessive degenerative or necrotic changes and to select for a dose dependent development of proliferative, metaplastic or dysplastic changes.

Two serial sections approximately 300 μm apart were made tissue from blocks containing explants collected at 3 weeks from the definitive study. Each section was qualitatively evaluated and results compared and each histomorphometrically analyzed. Every explant containing a metaplastic, dysplastic or hyperplastic lesion of the mucosa was photographed and the image digitized into a computer coupled image analysis system. The total cross-sectional area of each mucosa was determined and the total area within the mucosa containing one of the above tissue changes (altered differentiation) was determined and the percent mucosal area of altered epithelium (as defined above) was calculated. The values from each serial section were averaged and group means with standard deviations were calculated. The data were statistically evaluated by non-parametric analysis of variance techniques and pair-wise comparisons were made by a t-test which makes allowance for unequal variance.

3. RESULTS

All results of these studies were based upon the morphologic appearance of the tracheal explants following either 1 or 3 weeks in tissue culture. Results from the range-finding study were limited to qualitative evaluations. Lesions were classified based on histopathologic criteria and graded according to distribution and severity. Descriptive narratives of each major lesion is provided below and summary tables of lesion incidence and severity by test article and concentration are also included.

Similar qualitative histopathologic evaluations were conducted for each explant collected from the definitive study. In addition, mucosal lesions which were considered to be pre-neoplastic (metaplasia, hyperplasia or dysplasia) were quantitated by image analysis from explants collected after 3 weeks of culture. There were unequal numbers of explants in each group due to a variable but limited loss of explants during the culture period or tissue processing.

3.1 Particle Characterization.

The particle size distribution of each test material was determined by either electrozone analysis or quantitative image analysis. Table 1 lists for each particle the mass median diameter (MMD) and geometric standard deviation (GSD), the count median diameter (CMD) the aspect ratio (Length: Width) where applicable and the percent silica in each sample. Detailed particle size analysis data with cumulative values for both mass and frequency are in Appendix A. The MMD of the 10 particles ranges from 2.0 μm for asbestos to 44.8 μm for the iron whiskers. The iron whiskers that were initially received for this study were large and fibrous. In order to suspend the material in media and expose the tissue cultures, the iron whiskers were ground into a finer powder. The grinding succeeded in breaking apart the fibers, yielding short cuboidal particles that had large diameters. Aspect ratios were not calculated for the ground material since the length of the particles approximated the width (measured by the electrozone analysis method). This was confirmed by observation of light microscopic preparations of the ground test article.

The silica content of each test particle is also presented in Table 1. The percent silica of each material was less than 1 percent except for the glass beads and asbestos which had 68.5 percent and 48.1 percent silica, respectively.

3.2 Range-Finding Study.

The majority of tracheal explants cultured in media alone retained normal architectural features through 3 weeks. The predominant morphological change in control explants was a shift in the normal pseudostratified columnar epithelium to a cuboidal or simple squamous layer. There was no evidence of any keratinization and surface membrane structures such as microcillia were often visible. The incidence of this change (reduced epithelial height) was much higher in the 3-week samples compared to 1-week explants. The severity of the change was generally mild to occasionally moderate.

TABLE 1. PARTICLE SIZE DISTRIBUTION AND SILICA CONTENT OF MINERAL PARTICLES

Test Material	MMD	GSD	CMD	Aspect Ratio	Percent Silica
Glass Beads	2.4	1.3	2.0	NA	68.50
Asbestos	2.0	3.3	0.1	12.3	48.10
Graphite Fibers	10.4	1.5	--	3.3	2.46
KS-2 Graphite	4.1	1.9	1.2	NA	0.06
Micro-260 Graphite	3.7	1.8	1.3	NA	0.31
Printex L-Carbon Black	16.1	2.5	1.7	NA	< 0.01
Aluminum Dust	4.9	2.2	1.0	NA	0.03
Iron Whiskers	44.8	1.7	18.7	NA	0.15
Brass Dust	4.3	2.3	0.6	NA	0.06
Ni-Graphite	10.9	1.5	--	2.9	0.89

NA - Not Applicable

The second most frequent epithelial change that was observed in control tissues was mucosal degeneration and occasional focal necrosis. The incidence summary tables combines both degenerative and necrotic lesions. Both were considered indicative of non-specific cytotoxicity and for the purposes of these studies, the grading of degenerative and necrotic changes was based on distribution and severity. Focal to multifocal, hydropic vacuolization or cytomegaly with an eosinophilic ground glass appearance was considered mild degeneration. A diffuse distribution of similar changes or focal cell death was graded as a moderate lesion. Severe lesions were characterized by multifocal to diffuse coagulation necrosis of the mucosal epithelium or widespread cellular desquamation. There was a 40 percent incidence of mild degeneration in the media control explants at one week and a

15 percent incidence of mild degenerative changes after 3 weeks in cultures. The decrease in lesion incidence likely represents repair of mucosal epithelium which was damaged as a result of tissue culture manipulation. The only remaining finding among control explants was occasional mild to moderate hyperplasia. The lesions were focal and were more numerous in the 1 week samples compared to the 3 week tissues. This may also reflect regeneration reparative processes.

The incidence of cytotoxic changes were relatively low in explants exposed to glass beads. Lesion occurrence and severity did not appear related to concentration of the negative control material. Mild cuboidal to squamous change generally occurred more frequently in the organ cultures collected 3 weeks after exposure. With few exceptions, mild degenerative changes were limited to the 3-week cultures. Mild focal hyperplasia occurred in 10 percent-20 percent of several groups with no apparent predilection for the one or three week sample periods. Finally, a single focus of squamous metaplasia was observed in the 100 µg /ml, 3 week collection group. Overall the explants exposed to glassbeads appeared similar to media control tracheal organ cultures and had incidences across all concentration groups of mild non-specific tissue changes that were comparable to media control tissues.

Tracheal organ cultures exposed to crocidolite asbestos had a noticeably higher incidence of cytotoxic lesions relative to media or negative control tissues at concentrations of 100 µg/ml or higher. Lesions involving alterations in cell growth or differentiation occurred at each concentration level, with increasing frequency with higher concentrations. Mild to moderate mucosal hyperplasia, often accompanied by basal cell hypertrophy was the most common change. Multifocal squamous metaplasia of the lumenal epithelium, with occasional keratinization was observed primarily in explants treated with 1, 10 or 100 mg/ml of asbestos and collected after 3 weeks of culture.

In the range-finding study, each of the three graphite materials (graphite fibers, KS-2 graphite and Micro-260 synthetic graphite) appeared to cause variable degrees of cytotoxic damage to tracheal mucosal epithelium, but each appeared to be comparable to glass beads in the incidence and severity of proliferative changes or disturbance in differentiation. Graphite fibers were least toxic, causing mild to moderate cuboidal to squamous change,

predominantly at concentrations of 10 mg/ml and 100 mg/ml. Explants exposed to 1 mg/ml or higher of graphite had mild degeneration. Mild mucosal hyperplasia and metaplasia were most predominant at the highest concentration (100 mg/ml).

Cytotoxic lesions were generally limited to mild cuboidal to squamous change across all dose groups for KS-2 graphite and mild degeneration at concentrations of 1 mg/ml and higher. Moderate cytotoxic lesions were occasionally noted in tracheal explants treated with micro-260 graphite. Mucosal hyperplasia, dysplasia and squamous metaplasia were also mild in severity, focally distributed and were relatively limited in incidence to the higher concentrations for both compounds.

Printex L-carbon black caused a dose-dependent incidence of mild to moderate, with occasionally severe, squamous change, degeneration and necrosis. The most frequent and more severe changes occurred at levels of 1 mg/ml and higher. Carbon black caused no significant hyperplastic or metaplastic changes at any of the concentrations tested. Mild to moderate epithelial dysplasia did occur with some frequency in 3 week cultures at levels of 10 µg/ml and higher.

Aluminum dust caused approximately 20 percent-60 percent incidence of cytotoxic lesions of a mild to moderate nature across all dose groups, with higher frequency occurring at the 100 µg/ml-100 mg/ml dose levels. The only significant occurrence of other changes was mild to moderate mucosal hyperplasia after exposure to 10 mg/ml or 100 mg/ml of aluminum dust.

Brass dust caused severe degeneration and necrosis in tracheal organ cultures exposed to concentrations of 1 mg/ml or higher. Mild to moderate cuboidal to squamous change and multifocal degeneration occurred in all lower dose groups. There were no proliferative changes in the higher dose groups due to the overt toxicity, however, the incidence of mucosal hyperplasia and dysplasia appeared slightly greater at concentrations of 10 µg/ml and 100 µg/ml than observed in control.

There was a similar dose-relationship in mucosal degeneration and cell death following exposure to both polycrystalline iron whiskers and nickel-coated graphite fibers. Each test material caused complete epithelial desquamation at the highest concentration (100 mg/ml). There was a variable

incidence of hyperplastic, metaplastic and dysplastic changes following exposure to lower, less cytotoxic concentrations of each test material.

3.3 Definitive Study.

The percent of tracheal organ cultures bearing morphologic changes is listed in Table 2. Many explants contained more than one lesion, and therefore, the values do not equal 100 percent.

Based upon the observations in the range finding study, it appeared that concentrations of 100 µg/ml, 1 mg/ml and 10 mg/ml would be suitable levels for each test article, with two exceptions. These concentrations of brass dust and Ni-coated graphite caused excessive cytotoxicity. Therefore, in order to properly assess the potential of each material to cause disturbances in cell growth or differentiation, levels of 10 µg/ml, 100 µg/ml and 1 mg/ml were selected for these two materials and a fourth concentration of 1 µg/ml was added for brass dust to ensure survival of a group of exposed explants for morphometric analysis. It was apparent, however, from the preliminary study results that it would be difficult to quantitatively compare each test material, at a single concentration that produced some degree of proliferative change.

Qualitatively, there were few differences in the incidence, distribution, or severity of lesions between explants exposed to similar concentrations of each test material during the range-finding and definitive studies. More explants per dose level and collection time point were incorporated in the definitive study, providing a larger data base.

Most media control explants were normal after 1 week of culture and showed mild cuboidal changes or degeneration after 3 weeks. Mild to moderate focal squamous metaplasia was noted in 4 of 15 explants collected after 3 weeks of culture.

The morphological appearance of explants exposed to glass beads was similar to the media control tissues. Mild focal degeneration or loss of epithelial height were the predominant findings. Few changes in differentiation or architecture were observed in the negative control groups after 1 week. Mild hyperplasia and squamous metaplasia were observed in several tracheal organ cultures collected at 3 weeks.

Explants exposed to crocidolite asbestos had significant incidences of proliferative or cellular differentiation changes. There were 67 percent, 73 percent, and 86 percent incidences of disturbances in cell growth or differentiation in explants cultured for 3 weeks and exposed to the 100 µg/ml, 1 mg/ml and 10 mg/ml groups, respectively (Table 2). Degenerative changes, loss of epithelial height, and mucosal hyperplasia were the most prevalent changes in explants collected 1 week after exposure.

Explants exposed to the various graphite materials (graphite fibers, KS-2 graphite and Micro-260 graphite) had mild to moderate cytotoxic changes that generally increased in frequency with higher concentrations. Although, Micro-260 graphite caused a consistent incidence of cuboidal to squamous change across each dose level, none of the concentrations tested of these three graphite materials appeared to produce a notable incidence of disturbances in cell growth or differentiation, with the exception of explants exposed to 1 mg/ml for both KS-2 graphite and Micro-260 and collected after 3 weeks.

Printex L-carbon black and aluminum produced changes similar to those observed in the range-finding study. There was a high incidence of mild to moderate cuboidal to squamous change 3 weeks after exposure. Mild squamous metaplasia was the only other significant change that occurred in explants exposed to 100 µg/ml and 1 mg/ml of carbon black and collected at 3 weeks. Mucosal dysplasia was present in approximately half of the explants of the two higher dose groups treated with aluminum dust.

Brass dust was again extremely cytotoxic at concentrations of 100 µg/ml and higher. Less severe degenerative changes were prevalent down to the 1 µg/ml level. There were no notable hyperplastic, metaplastic or dysplastic changes.

Explants exposed to iron whiskers had a higher incidence of cytotoxic lesions at the lowest dose (100 µg/ml) than the nigher doses, suggesting that the changes were not related to exposure to the test article but were likely due to a deficiency in culture conditions for that group.

TABLE 2. PERCENT OF TRACHEAL ORGAN CULTURES WITH MUCOSAL LESIONS - DEFINITIVE STUDY[1]

	Media Control	Glass Beads	Asbestos	Printex L	Graphite Fibers	KS-2 Graphite	Micro 260	Iron Whiskers	Aluminum Dust	Brass Dust	Ni-Graphite
						100 µg/ml					
Cytotoxic Lesions	28/47	31/36	53/40	87/69	71/NA	50/60	80/100	86/100	86/31	100/100	87/92
Proliferative or Cellular Differentiation	8/27	0/14	20/67	33/69	14/NA	36/47	40/29	0/0	43/69	17/50	27/77
						1 mg/ml					
Cytotoxic Lesions	28/47	86/80	7/20	85/71	40/62	60/73	80/71	64/20	73/38	100/100	100/100
Proliferative or Cellular Differentiation	8/27	0/40	50/73	15/71	40/38	7/73	13/64	7/87	40/77	0/0	0/29
						10 mg/ml					
Cytotoxic Lesion	28/47	33/29	75/29	73/100	67/92	67/73	67/60	87/13	100/73	NA	NA
Proliferative or Cellular Differentiation Lesions	8/27	20/71	38/86	13/13	13/54	13/47	13/33	33/87	20/53	NA	NA

[1]Values are the percent of total explants in a group having a cytotoxic lesion (cuboidal squamous change, a degeneration or necrosis) or disturbances in cell growth or differentiation (hyperplasia, metaplasia, or dysplasia). The first value listed is for cultures collected 1 week after exposure, the second value is for explants collected 3 weeks after exposure.

NA - Not available. A 10 mg/ml dose level was not included for Brass dust or Ni-graphite due to overt toxicity. The 3 weeks, 100 µg/ml group of graphite fiber exposed tracheas were removed from study due to bacterial contaminatin.

Eighty-seven percent of explants treated with 1 mg/ml or 10 mg/ml of iron whiskers developed proliferative or dedifferentiation lesions by 3-weeks post-exposure. Most of these lesions were mild in severity.

Nickel-coated graphite was also markedly cytotoxic, causing diffuse necrosis at the 1 mg/ml level. The incidence of proliferative, metaplastic and dysplastic-changes was inversely proportional to the incidence and severity of degenerative and necrotic changes.

3.4 Morphometric Analysis.

The results of the image analysis studies of tracheal organ cultures are summarized in Table 3. The individual values for each serial determination are in Appendix C. The values listed in Table 3 are the mean percentage of each group of tracheal explant mucosas analyzed that had proliferative changes or alterations in cellular differentiation. Due to the unequal number of explants in each group (a result of explant death or loss unrelated to treatment) the data was nonhomogeneous. Therefore, nonparametric analysis of variance techniques were used to examine statistical differences between groups.

Among explants exposed to 100 µg/ml of each test article, both asbestos and Ni-coated graphite groups had a greater degree of lesion involvement ($p \leq 0.05$) compared to both media controls and explants exposed to glass beads (negative control). Explants exposed to brass dust also had a significantly greater area of tissue change compared to the glass bead group. Explants exposed to media alone, glass beads, KS-2 graphite, micro-260 graphite, and iron whiskers each had significantly less ($p \leq 0.05$) mucosal lesion development compared to asbestos (positive control).

Comparison of explants exposed to 1 mg/ml of each test material showed that explants treated with asbestos, KS-2 graphite, aluminum dust and iron whiskers had significantly ($p \leq 0.05$) more mucosal lesions than the media or negative control groups. Explants exposed to asbestos had an average of 32 percent mucosal involvement of a proliferative or metaplastic nature, which was significantly greater than the media, glass beads, brass dust and Ni-graphite groups. Explants in these later two groups treated with brass dust or Ni-graphite had moderate to occasionally severe degenerative and necrotic changes following treatment with concentrations of 1 mg/ml; a tissue condition which is not conducive to proliferative changes.

TABLE 3. GROUP MEAN PERCENTAGE OF TRACHEAL EXPLANT MUCOSA WITH HYPERPLASTIC, METAPLASTIC OR DYSPLASTIC LESIONS

Test Material	100 µg/ml	1 mg/ml	10 mg/ml
Media Control	10.1 ± 12.2[c]	10.1 ± 12.2[c]	10.1 ± 12.2[b,c]
Glass Beads	7.1 ± 9.5[c]	11.1 ± 14.7[c]	31.5 ± 27.4[a]
Asbestos	27.6 ± 26.2[a,b]	32.0 ± 24.4[a,b]	31.9 ± 22.4[a]
Graphite Fibers	NA	24.2 ± 25.7	21.8 ± 21.2
KS-2 Graphite	12.9 ± 18.6[c]	25.8 ± 27.8[a]	9.7 ± 11.9[b,c]
Micro-260 Graphite	11.1 ± 15.5[c]	21.0 ± 19.1	11.3 ± 14.5[b,c]
Printer L	13.7 ± 12.4	22.7 ± 16.9	10.9 ± 15.4[b,c]
Aluminum Dust	16.9 ± 14.8	38.4 ± 28.1[a,b]	18.7 ± 17.3[c]
Iron Whiskers	0.0 ± 0.0[c]	30.4 ± 21.0[a,b]	22.8 ± 14.8
Brass Dust	23.6 ± 25.6[b]	0.0 ± 0.0[c]	NI
Ni-Graphite	35.1 ± 27.2[a,b]	14.9 ± 25.3[c]	NI

[a]Significantly different ($p \leq 0.05$) from media control.
[b]Significantly different ($p \leq 0.05$) from negative control (glass beads).
[c]Significantly different ($p \leq 0.05$) from positive control (asbestos).
NA - group not available due to loss of cultures by bacterial contamination.
NI - group not included in experimental design.

Differences between groups began to diminish at 10 mg/ml level, apparently due to increased cytotoxic damage. Tracheal organ cultures exposed to asbestos, glass beads had a significantly ($p \leq 0.05$) higher percentage of lesion development compared to media controls with a suggested difference noted in explants exposed to iron whiskers. Each test article, except iron whiskers, glass beads and graphite fibers had significantly less mucosal reaction relative to asbestos. Explants were not exposed to 10 mg/ml of brass dust or Ni-coated graphite due to the severe necrosis that occurs at that concentration.

4. DISCUSSION

There are a number of in vitro models with a variety of endpoints that have been used to assess the carcinogenic potential of both organic and inorganic materials. The latter class of substances (which have low solubility rates) are proposed to exert their oncogenic effects by epigenetic mechanisms. Because these mechanisms typically involve direct physical contact with a target cell, organ culture systems are well suited to examine the neoplastic effects of inorganic materials. The tracheal explant model has been investigated for over 20 years with these objectives in mind, and techniques have evolved that allow development of putative neoplastic lesions in the mucosa following exposure to known carcinogens (i.e., asbestos); lesions which are morphologically and histiogenically similar to those observed in the upper airway mucosa of live animals and humans subsequent to carcinogen exposure.

The results of the studies presented in this report suggest that there are significant differences between the test particles in the type, incidence and severity of the epithelial changes that each induced after in vitro exposure. The spectrum and magnitude of differences were best appreciated by qualitative histopathological evaluations, but the combined proliferative and altered cellular differentiation changes were also measurable by quantitative histomorphometry. For example, the severe cytotoxicity of brass dust and Ni-coated graphite was easily determined by qualitative evaluation, while image analysis of tracheal explants identified statistically significant increases in pre-neoplastic lesions in tissues treated with asbestos, iron whiskers, Ni-coated graphite, aluminum dust or KS-2 graphite, at least, with one of the concentration tested.

There were several factors in this study which prevent direct comparisons of each test article at identical concentrations and, therefore, limit final interpretations. Perhaps the most important of these factors is the issue of delivered dose to the target tissue. Since it was the intent to examine these materials in a physical state that was similar to the physical nature of the material that may be involved in potential human exposure, there was little to no attempt to equalize particle size distributions between test

materials. The long fibrous materials were ground with a mortar and pestle to a fine powder in order to suspend the particles for explant exposure. However, the particle size distribution data showed there was a marked disparity between test materials, ranging from an MMD of 2.0 μm for asbestos to 44.8 μm for iron whiskers. The majority of the test particles had MMD of 10 μm or less, which should allow for evenly distributed cellular interaction and some internalization of particles. However, since in determining the MMD of a test material, mass is a cube function of diameter, a monodispersed particulate material with an MMD of 1 μm has 1000 times more particles in the range of the smaller fraction (~1 μm) than does a material with an MMD of 10 μm. Therefore, materials with smaller size distributions will have significantly more particles in the size range which would likely interact with a target cell. Therefore, the relative non-toxic behavior of Printer-L carbon black (MMD of 16.1) may be in part due to a lower delivered concentration of "reactive-size" particle to target cells. In contrast, the iron whiskers which had an MMD of 44.8 μm was one of the more reactive materials in this assay, and would likely be more toxic in this model system if the MMD were reduced.

The second feature which precludes direct comparison of each material at a specified concentration is the relative cytotoxic effects each test article demonstrated. The experiment was designed to assess the potential of each test article to induce changes in tissue differentiation or proliferative response in tracheal organ culture. Since cytotoxic changes (necrosis) generally preclude cell growth or dedifferentiation, non-cytotoxic concentrations of each material were selected to examine the former effects. The non-cytotoxic dose varied for each test substance. The less cytotoxic each material was, the incidence of proliferative changes increased with elevated concentrations. This effect was not noticeable with glass beads at 10 mg/ml; at which concentration each other particle induced over a two-thirds incidence of cytotoxic change, with corresponding decreases in proliferative lesions. However, 10 mg/ml of glass beads did not cause an increase in cell damage relative to media controls, but after 3 weeks in culture did increase the percentage of proliferative tissue changes.

One final complicating factor inherent in this test model is the spontaneous change of lumenal mucosa from tall columnar epithelium to a simple cuboidal or squamous layer. This effect was typically more prevalent with increased time in culture and appears to be a non-specific response to the environmental conditions of tissue culture. The change can also be induced (as it was in this study) by various toxic substances. However, the toxic response must be considered relative to the background changes in control tissues. These changes can also be minimized by addition of serum or retenoids to the culture media. However, the addition of serum or retenoids also protects the epithelium from metaplastic, dysplastic, and hyperplastic lesions which occur following exposure to materials like asbestos. Since the intent of these studies was to optimize conditions to permit development of these lesions, only a small, defined amount of retinyl acetate was added to the media, at a concentration sufficient to prevent spontaneous squamous metaplasia in control tissues, but does not prevent asbestos-induced metaplasia (results previously defined in the literature and this laboratory[3,8].

5. CONCLUSION

Therefore, given the limitations discussed above, the following is proposed as a relative rank ordering of the potential of each test material to induce morphologically apparent pre-neoplastic lesions in tracheal organ cultures. Crocidolite asbestos (positive control) and Ni-coated graphite were the most reactive in this study. Iron whiskers, aluminum dust and KS-2 graphite caused an intermediate level of tissue response, while the remaining substances did not cause significant tissue responses compared to control explants. The most cytotoxic materials were brass dust and Ni-coated graphite. Asbestos and iron whiskers caused significantly less cellular damage, but slightly more than the graphite materials, aluminum, carbon black or glass beads.

6. SPECIMEN STORAGE AND RECORD ARCHIVES

Records of animal receipt, quarantine, organ culture procedures, tissue exposure data, test article analysis, histopathology, morphometric analysis, and other information pertinent to the conduct of this study as defined by the protocol are contained in appropriate labeled notebooks. All original data and pathology materials are stored at Battelle-Columbus Division. The remaining test article will be returned to the Client subsequent to submission of the final report. Tissue samples will be returned to the Client upon approval of the final report.

7. ACKNOWLEDGEMENTS

The name, title, and degree of the persons participating in the conduct of this study are presented in the following list:

Principal Contributors

Name	Title	Degree
Dr. Michael Placke	Study Director	Ph.D.
Dr. Afaf Wensky	Chemist	Ph.D.
Mr. David Long	Technician	B.S.
Ms. Roberta Smith	Histology Supervisor	B.S.
Dr. Douglas Craig	Program Manager	Ph.D.
Dr. Gerald Fisher	Department Manager	Ph.D.

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APPENDIX A

PARTICLE SIZE DISTRIBUTION ANALYSIS AND SILICA DETERMINATION DATA

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PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 20 DEC 85 :DATA
SAMPLE: GLASS BEADS I-9742 :JOB NUMBER

VOLUME (MASS) DISTRIBUTION FROM DISPLAY AREA: 4

INDICES

VOLUME MODE = 2.79 MEDIAN = 2.60 MICRONS AND LARGER

GEOMETRIC VOLUME MEAN = 2.54 +/- .72 (28.28%) SKEWNESS = -.36

ARITHMETIC VOLUME MEAN = 2.61 +/- .63 (23.95%) SKEWNESS = -.29

PERCENTILE:	00.1%	OF VOLUME IS AT	5.58 MICRONS AND LARGER
PERCENTILE:	01.0%	OF VOLUME IS AT	4.23 MICRONS AND LARGER
PERCENTILE:	06.0%	OF VOLUME IS AT	3.56 MICRONS AND LARGER
PERCENTILE:	22.0%	OF VOLUME IS AT	3.10 MICRONS AND LARGER
PERCENTILE:	50.0%	OF VOLUME IS AT	2.60 MICRONS AND LARGER
PERCENTILE:	78.0%	OF VOLUME IS AT	2.12 MICRONS AND LARGER
PERCENTILE:	94.0%	OF VOLUME IS AT	1.66 MICRONS AND LARGER
PERCENTILE:	99.0%	OF VOLUME IS AT	1.26 MICRONS AND LARGER
PERCENTILE:	99.9%	OF VOLUME IS AT	.99 MICRONS AND LARGER

COUNT (FREQUENCY) DISTRIBUTION FROM DISPLAY AREA: 5

INDICES

COUNTS MODE = 2.27 MEDIAN = 2.12 MICRONS AND LARGER

GEOMETRIC COUNTS MEAN = 2.03 +/- .70 (34.29%) SKEWNESS = -.33

ARITHMETIC COUNTS MEAN = 2.12 +/- .61 (28.52%) SKEWNESS = -.24

PERCENTILE:	00.1%	OF COUNTS IS AT	4.38 MICRONS AND LARGER
PERCENTILE:	01.0%	OF COUNTS IS AT	3.56 MICRONS AND LARGER
PERCENTILE:	06.0%	OF COUNTS IS AT	3.10 MICRONS AND LARGER
PERCENTILE:	22.0%	OF COUNTS IS AT	2.60 MICRONS AND LARGER
PERCENTILE:	50.0%	OF COUNTS IS AT	2.12 MICRONS AND LARGER
PERCENTILE:	78.0%	OF COUNTS IS AT	1.60 MICRONS AND LARGER
PERCENTILE:	94.0%	OF COUNTS IS AT	1.26 MICRONS AND LARGER
PERCENTILE:	99.0%	OF COUNTS IS AT	.99 MICRONS AND LARGER
PERCENTILE:	99.9%	OF COUNTS IS AT	.89 MICRONS AND LARGER

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS   20 DEC 85 :DATA
SAMPLE: GLASS BEADS   I-9742 :JOB NUMBER

PARTICLE SIZE VS. COUNTS
ENCLOSING
LOW AT 18 .89 112 HIGH AT 74 6.20 1

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL COUNTS FROM CHANNEL 18 TO 74, AND SKIP: 2

% MAX	SIZE
.0	.83
4.6	.92
11.5	1.02
20.1	1.13
31.3	1.26
46.7	1.40
59.5	1.55
78.9	1.72
89.1	1.91
96.5	2.12
99.7	2.35
88.9	2.60
67.4	2.89
36.7	3.21
11.1	3.56
3.1	3.95
.7	4.38
.2	4.86
.2	5.39
.0	5.98

Scale: 0 10 20 30 40 50 60 70 80 90 100

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 20 DEC 85 :DATA
SAMPLE: GLASS BEADS I-9742 :JOB NUMBER

"TOTAL IN TABULATION- TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE 20 DEC
SIZE-NORMALIZED COUNTS DISTRIBUTION
TOTAL = 91563

CHNL	SIZE	COUNTS	% >	CHNL	SIZE	COUNTS	% >	CHNL	SIZE	COUNTS	% >
18	.89	112	100.00	37	1.72	3231	73.61	56	3.32	1059	3.42
19	.92	187	99.88	38	1.78	3427	70.08	57	3.44	744	2.26
20	.95	271	99.67	39	1.84	3498	66.33	58	3.56	453	1.45
21	.99	367	99.38	40	1.91	3651	62.51	59	3.68	324	.95
22	1.02	469	98.98	41	1.97	3728	58.53	60	3.81	220	.60
23	1.06	555	98.46	42	2.04	3849	54.46	61	3.95	126	.36
24	1.10	696	97.86	43	2.12	3954	50.25	62	4.09	60	.22
25	1.13	823	97.10	44	2.19	4059	45.93	63	4.23	47	.16
26	1.17	933	96.20	45	2.27	4095	41.50	64	4.38	29	.10
27	1.22	1066	95.18	46	2.35	4062	37.03	65	4.54	20	.07
28	1.26	1281	94....	47	2.43	3947	32.57	66	4.70	17	.05
29	1.30	1416	92.62	48	2.52	3875	28.26	67	4.86	10	.03
30	1.35	1609	91.07	49	2.60	3642	24.03	68	5.03	4	.02
31	1.40	1963	89.31	50	2.70	3482	20.05	69	5.21	4	.02
32	1.45	2054	87.22	51	2.79	3149	16.25	70	5.39	2	.01
33	1.50	2246	84.98	52	2.89	2761	12.81	71	5.58	1	.00
34	1.55	2439	82.53	53	2.99	2359	9.79	72	5.78	1	.00
35	1.60	2728	79.86	54	3.10	1974	7.22	73	5.98	1	.00
36	1.66	3000	76.88	55	3.21	1505	5.06	74	6.20	1	.00

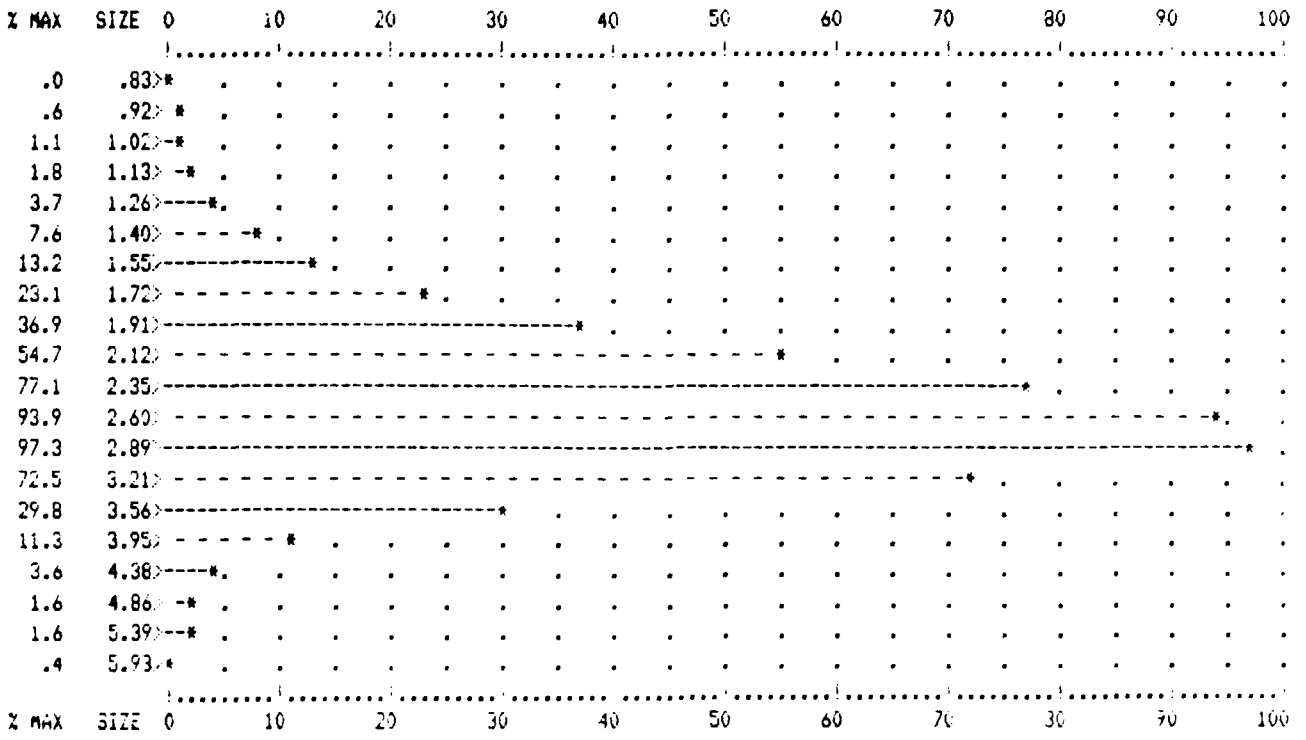
DISPLAY AREA: 4

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 MAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 20 DEC 83 :DATA
SAMPLE: GLASS BEADS I-9742 :JOB NUMBER

PARTICLE SIZE VS. VOLUME
ENCLOSING
LOW AT 18 .89 75 HIGH AT 74 6.20 318

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL VOLUME FROM CHANNEL 18 TO 74, AND SKIP: 2



PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 20 DEC 85 :DATA
SAMPLE: GLASS BEADS I-9742 :JOB NUMBER

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE 20 DEC
SIZE-NORMALIZED VOLUME DISTRIBUTION
TOTAL = 1049654

CHNL	SIZE	VOLUME	% >	CHNL	SIZE	VOLUME	% >	CHNL	SIZE	VOLUME	% >
18	.89	75	100.00	37	1.72	15138	93.48	56	3.32	37065	13.09
19	.92	408	99.99	38	1.78	16456	92.04	57	3.44	26887	9.56
20	.95	478	99.95	39	1.84	20705	90.28	58	3.56	19540	6.81
21	.99	560	99.91	40	1.91	24204	88.29	59	3.68	15476	4.93
22	1.02	738	99.85	41	1.97	27420	85.99	60	3.81	11695	3.48
23	1.06	873	99.78	42	2.04	31427	83.37	61	3.95	7392	2.36
24	1.10	97_	99.70	43	2.12	35819	80.38	62	4.09	3919	1.65
25	1.13	1147	99.61	44	2.19	40796	76.97	63	4.23	3438	1.28
26	1.17	1444	99.50	45	2.27	45670	73.08	64	4.38	2356	.96
27	1.22	18_9	99.36	46	2.35	50518	68.73	65	4.54	1743	.73
28	1.26	2439	99.19	47	2.43	54195	63.92	66	4.70	165_	.57
29	1.30	2995	98.95	48	2.52	59028	58.75	67	4.86	1073	.41
30	1.35	3772	98.67	49	2.60	61556	53.13	68	5.03	510	.31
31	1.40	4977	98.31	50	2.70	65319	47.27	69	5.21	5oo	.26
32	1.45	5928	97.83	51	2.79	65535	41.04	70	5.39	1047	.20
33	1.50	7194	97.27	52	2.89	63759	34.80	71	5.58	232	.18
34	1.55	8667	96.58	53	2.99	60450	28.73	72	5.78	258	.06
35	1.60	10755	95.76	54	3.10	56126	22.97	73	5.98	265	.06
36	1.66	13124	94.73	55	3.21	47463	17.62	74	6.20	318	.03

*

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE 16 JAN 85 :DATA
SAMPLE: CROCIDOLITE I-9742 :JOB NUMBER

VOLUME (MASS) DISTRIBUTION FROM DISPLAY AREA: 4
==========
INDICES

VOLUME MODE = 1.84 MEDIAN = 1.84 MICRONS AND LARGER

GEOMETRIC VOLUME MEAN = 1.92 +/- 5.28 (274.44%) SKEWNESS = .02

ARITHMETIC VOLUME MEAN = 4.59 +/- 7.74 (168.76%) SKEWNESS = .35

PERCENTILE: 00.1% OF VOLUME IS AT 67.71 MICRONS AND LARGER
PERCENTILE: 01.0% OF VOLUME IS AT 38.89 MICRONS AND LARGER
PERCENTILE: 06.0% OF VOLUME IS AT 18.14 MICRONS AND LARGER
PERCENTILE: 22.0% OF VOLUME IS AT 5.21 MICRONS AND LARGER
PERCENTILE: 50.0% OF VOLUME IS AT 1.84 MICRONS AND LARGER
PERCENTILE: 78.0% OF VOLUME IS AT .70 MICRONS AND LARGER
PERCENTILE: 94.0% OF VOLUME IS AT .25 MICRONS AND LARGER
PERCENTILE: 99.0% OF VOLUME IS AT .11 MICRONS AND LARGER
PERCENTILE: 99.9% OF VOLUME IS AT .07 MICRONS AND LARGER

COUNT (FREQUENCY) DISTRABUTION FROM DISPLAY AREA: 5
==========
INDICES

COUNTS MODE = .07 MEDIAN = .10 MICRONS AND LARGER

GEOMETRIC COUNTS MEAN = .12 +/- .08 (69.56%) SKEWNESS = .63

ARITHMETIC COUNTS MEAN = .14 +/- .13 (93.43%) SKEWNESS = .57

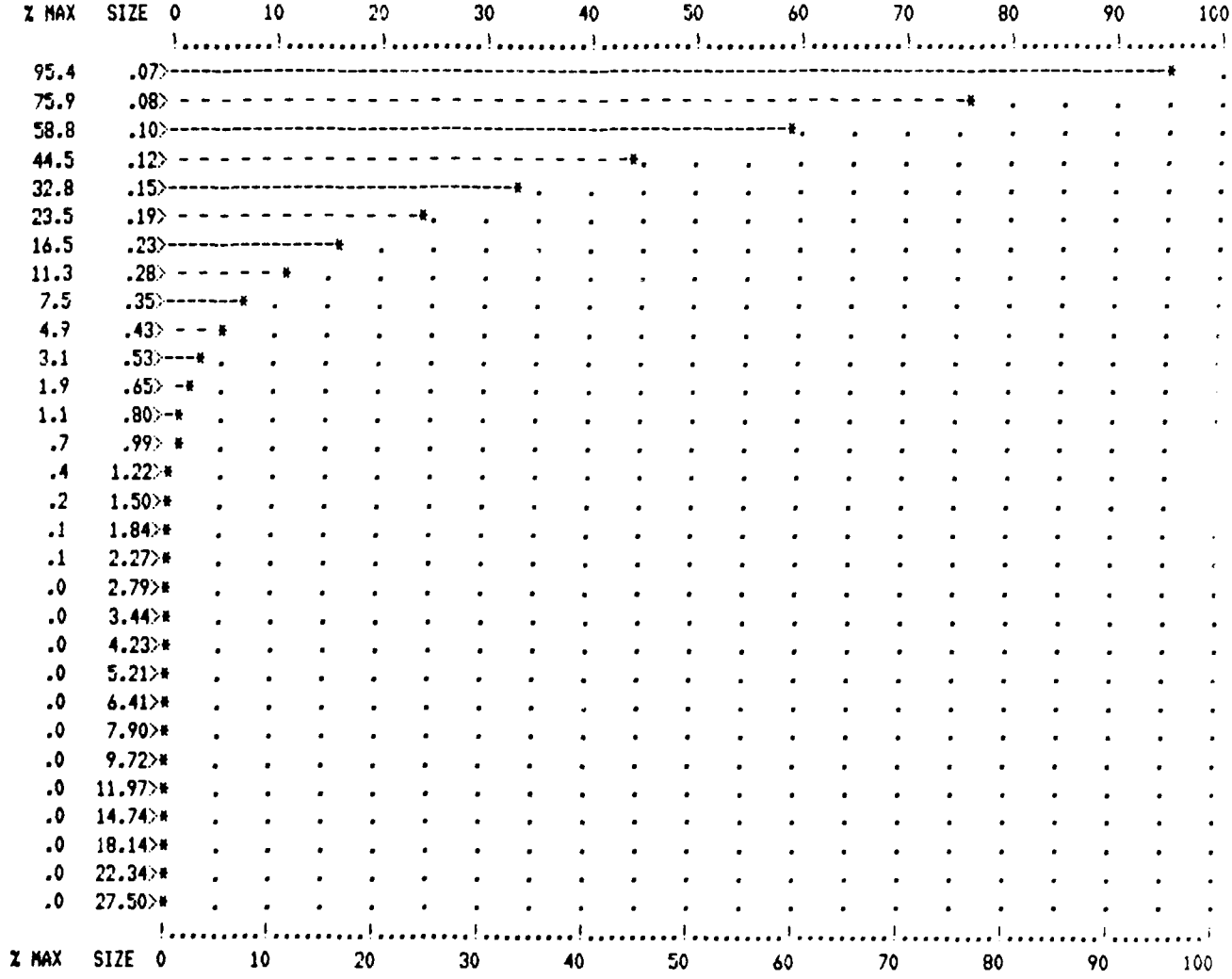
PERCENTILE: 00.1% OF COUNTS IS AT 1.50 MICRONS AND LARGER
PERCENTILE: 01.0% OF COUNTS IS AT .65 MICRONS AND LARGER
PERCENTILE: 06.0% OF COUNTS IS AT .30 MICRONS AND LARGER
PERCENTILE: 22.0% OF COUNTS IS AT .16 MICRONS AND LARGER
PERCENTILE: 50.0% OF COUNTS IS AT .10 MICRONS AND LARGER
PERCENTILE: 78.0% OF COUNTS IS AT .08 MICRONS AND LARGER
PERCENTILE: 94.0% OF COUNTS IS AT .07 MICRONS AND LARGER
PERCENTILE: 99.0% OF COUNTS IS AT .07 MICRONS AND LARGER
PERCENTILE: 99.9% OF COUNTS IS AT .07 MICRONS AND LARGER

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE 16 JAN 85 :DATA
SAMPLE: CROCIDOLITE I-9742 :JOB NUMBER

PARTICLE SIZE VS. COUNTS
ENCLOSING
LOW AT 1 .07 8000000 HIGH AT 88 27.50 1

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL COUNTS FROM CHANNEL 1 TO 88, AND SKIP: 2



PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE 16 JAN 85 :DATA
SAMPLE: CROCIDOLITE I-9742 :JOB NUMBER

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE NO DATE
SIZE-NORMALIZED COUNTS DISTRIBUTION
TOTAL =86890352

CHNL	SIZE	COUNTS	% >	CHNL	SIZE	COUNTS	% >	CHNL	SIZE	COUNTS	% >
1	.07	8000000	100.00	31	.53	257827	1.87	61	4.23	587	.00
2	.07	7434735	90.79	32	.57	220094	1.57	62	4.54	457	.00
3	.08	6889216	82.24	33	.61	187335	1.32	63	4.86	352	.00
4	.08	6365400	74.31	34	.65	158995	1.10	64	5.21	266	.00
5	.09	5864701	66.98	35	.70	134558	.92	65	5.58	195	.00
6	.09	5388074	60.23	36	.75	113553	.76	66	5.98	149	.00
7	.10	4936195	54.03	37	.80	95558	.63	67	6.41	117	.00
8	.11	4509419	48.35	38	.86	80097	.52	68	6.87	90	.00
9	.12	4107876	43.16	39	.92	66773	.43	69	7.37	69	.00
10	.12	3731526	38.43	40	.99	55206	.35	70	7.90	54	.00
11	.13	3379216	34.14	41	1.06	45777	.29	71	8.46	42	.00
12	.14	3052769	30.25	42	1.13	37657	.24	72	9.07	33	.00
13	.15	2749788	26.74	43	1.22	30808	.19	73	9.72	25	.00
14	.16	2469783	23.57	44	1.30	25298	.16	74	10.42	20	.00
15	.17	2211977	20.73	45	1.40	20937	.13	75	11.17	16	.00
16	.19	1975469	18.18	46	1.50	17288	.11	76	11.97	13	.00
17	.20	1759262	15.91	47	1.60	14286	.09	77	12.83	10	.00
18	.21	1562283	13.89	48	1.72	11859	.07	78	13.75	8	.00
19	.23	1383439	12.09	49	1.84	9665	.06	79	14.74	6	.00
20	.25	1221602	10.50	50	1.97	7830	.04	80	15.79	5	.00
21	.26	1075382	9.09	51	2.12	6369	.04	81	16.93	4	.00
22	.28	944368	7.85	52	2.27	5148	.03	82	18.14	3	.00
23	.30	826893	6.76	53	2.43	4145	.02	83	19.44	2	.00
24	.33	721955	5.81	54	2.60	3344	.02	84	20.84	2	.00
25	.35	628543	4.98	55	2.79	2665	.01	85	22.34	1	.00
26	.37	545666	4.26	56	2.99	2062	.01	86	23.94	1	.00
27	.40	472376	3.63	57	3.21	1626	.01	87	25.66	1	.00
28	.43	407774	3.09	58	3.44	1283	.01	88	27.50	1	.00
29	.46	351012	2.62	59	3.68	1007	.00				
30	.49	301296	2.21	60	3.95	778	.00				

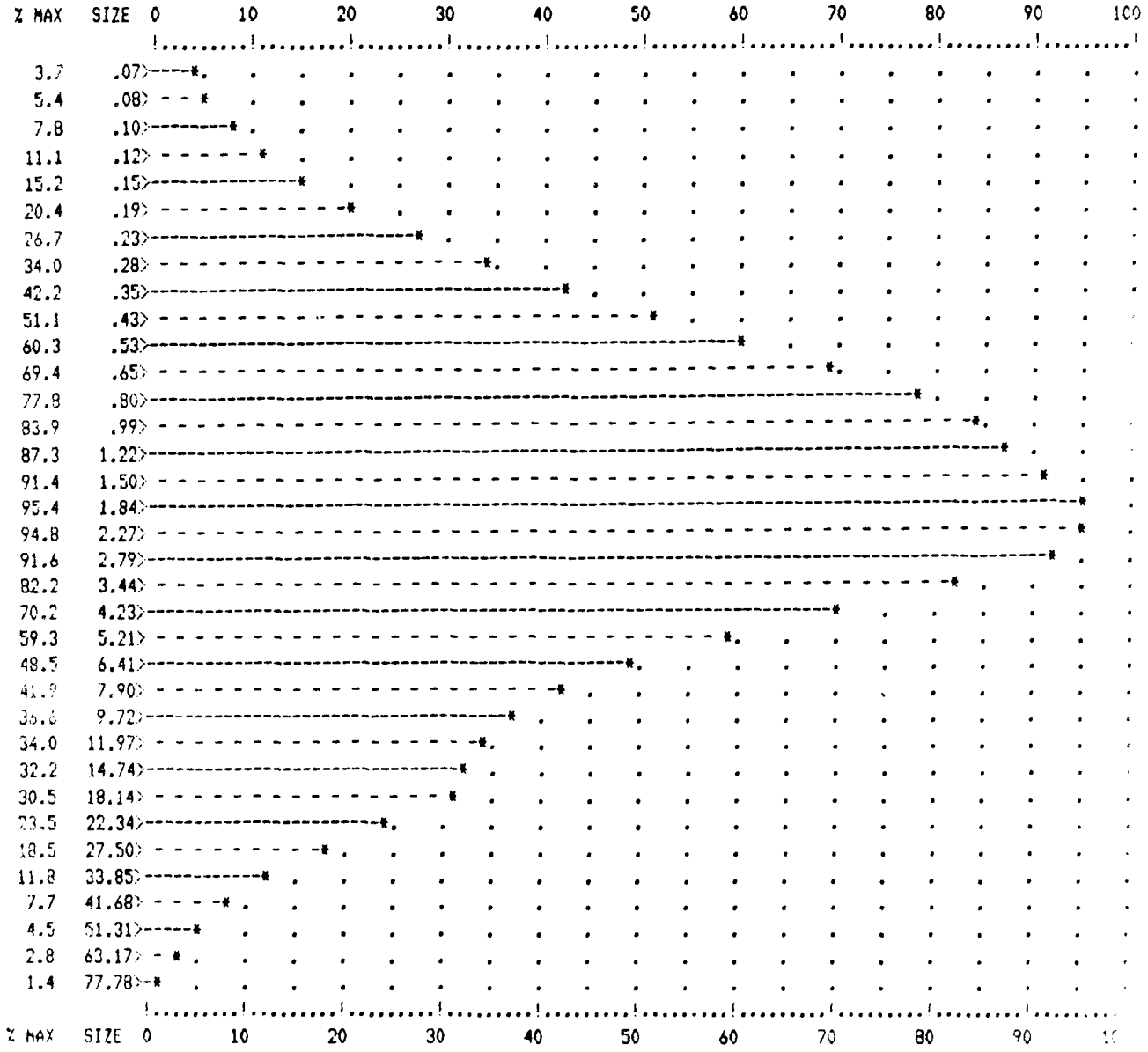
DISPLAY AREA: 4

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE 16 JAN 85 :DATA
SAMPLE: CROCIDOLITE I-9742 :JOB NUMBER

PARTICLE SIZE VS. VOLUME
ENCLOSING
LOW AT 1 .07 153223 HIGH AT 105 89.34 11493

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL VOLUME FROM CHANNEL 1 TO 105, AND SKIP: 2



PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE - 15 DAY RUN DATA
SAMPLE: CROCIDOLITE I-9742 JOB NUMBER

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE NO DATE
SIZE-NORMALIZED VOLUME DISTRIBUTION
TOTAL =85464179

CHNL	SIZE	VOLUME	% >	CHNL	SIZE	VOLUME	% >	CHNL	SIZE	VOLUME	% >
1	.07	153223	100.00	36	.75	3148773	76.50	71	8.46	1683089	14.75
2	.07	175283	99.91	37	.80	3262224	74.80	72	9.07	1621335	13.34
3	.08	199952	99.83	38	.86	3366449	73.04	73	9.72	1534803	12.97
4	.08	227447	99.72	39	.92	3455173	71.23	74	10.42	1478477	12.14
5	.09	257993	99.59	40	.99	3516928	69.37	75	11.17	1431614	11.34
6	.09	291312	99.45	41	1.06	3590360	67.47	76	11.97	1425911	10.67
7	.10	329133	99.30	42	1.13	3636151	65.53	77	12.83	1418116	9.80
8	.11	370176	99.12	43	1.22	3662400	63.57	78	13.75	1401141	9.04
9	.12	415160	98.92	44	1.30	3702522	61.60	79	14.74	1352193	8.28
10	.12	464294	98.70	45	1.40	3772598	59.60	80	15.79	1305759	7.65
11	.13	517773	98.44	46	1.50	3835045	57.57	81	16.93	1304148	6.85
12	.14	575782	98.17	47	1.60	3901628	55.50	82	18.14	1281154	6.15
13	.15	638477	97.86	48	1.72	3987273	53.40	83	19.44	1206238	5.46
14	.16	705999	97.51	49	1.84	4000000	51.25	84	20.84	1070037	4.81
15	.17	778453	97.13	50	1.97	3988796	49.09	85	22.34	985996	4.23
16	.19	855915	96.71	51	2.12	3996612	46.94	86	23.94	936355	3.70
17	.20	938425	96.25	52	2.27	3976624	44.78	87	25.66	908091	3.19
18	.21	1025978	95.74	53	2.43	3942575	42.64	88	27.50	775579	2.80
19	.23	1118529	95.19	54	2.60	3914602	40.51	89	29.47	698405	2.28
20	.25	1215980	94.59	55	2.79	3840687	38.40	90	31.59	612256	1.91
21	.26	1318184	93.93	56	2.99	3658884	36.33	91	33.85	496098	1.58
22	.28	1424937	93.22	57	3.21	3553173	34.26	92	36.28	414948	1.31
23	.30	1535981	92.45	58	3.44	3449260	32.44	93	38.89	354326	1.09
24	.33	1650994	91.62	59	3.68	3335165	30.58	94	41.68	322100	.90
25	.35	1769604	90.73	60	3.95	3168827	28.79	95	44.67	256151	.72
26	.37	1891369	89.78	61	4.23	2944275	27.09	96	47.88	215774	.58
27	.40	2015797	88.76	62	4.54	2826072	25.49	97	51.31	190187	.47
28	.43	2142336	87.67	63	4.86	2678056	23.97	98	55.00	168627	.36
29	.46	2270381	86.52	64	5.21	2480190	22.52	99	58.94	136838	.27
30	.49	2399275	85.29	65	5.59	2249446	21.18	100	63.17	117856	.20
31	.53	2528316	84.00	66	5.98	2122049	19.97	101	67.71	83181	.14
32	.57	2656763	82.64	67	6.41	2036123	18.82	102	72.57	58407	.09
33	.61	2783842	81.20	68	6.87	1939058	17.73	103	77.78	58084	.06
34	.65	2908753	79.70	69	7.37	1822335	16.68	104	83.36	42119	.03
35	.70	3030672	78.13	70	7.90	1755968	15.70	105	89.34	11493	.01

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
.55 MAIN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5858

DIEM: BATTELLE COLUMBUS LABS 20 DEC 85 :DATA
SAMPLE: DIESEL NATURAL GRAPHITE I-4741 :JOB NUMBER

VOLUME (MASS) DISTRIBUTION FROM DISPLAY AREA: 4

INDICES

VOLUME MODE = 4.54 MEDIAN = 4.23 MICRONS AND LARGER

GEOMETRIC VOLUME MEAN = 4.19 +/- 3.33 (79.47%) SKEWNESS = -.10

ARITHMETIC VOLUME MEAN = 5.00 +/- 3.43 (68.54%) SKEWNESS = .14

PERCENTILE: 00.1% OF VOLUME IS AT 20.00 MICRONS AND LARGER
PERCENTILE: 01.0% OF VOLUME IS AT 19.44 MICRONS AND LARGER
PERCENTILE: 06.0% OF VOLUME IS AT 11.17 MICRONS AND LARGER
PERCENTILE: 22.0% OF VOLUME IS AT 5.98 MICRONS AND LARGER
PERCENTILE: 50.0% OF VOLUME IS AT 4.23 MICRONS AND LARGER
PERCENTILE: 78.0% OF VOLUME IS AT 2.89 MICRONS AND LARGER
PERCENTILE: 94.0% OF VOLUME IS AT 1.72 MICRONS AND LARGER
PERCENTILE: 99.0% OF VOLUME IS AT .86 MICRONS AND LARGER
PERCENTILE: 99.9% OF VOLUME IS AT .51 MICRONS AND LARGER

COUNT (FREQUENCY) DISTRIBUTION FROM DISPLAY AREA: 4

INDICES

COUNTS MODE = .46 MEDIAN = .94 MICRONS AND LARGER

GEOMETRIC COUNTS MEAN = 1.11 +/- 1.06 (95.78%) SKEWNESS = .61

ARITHMETIC COUNTS MEAN = 1.41 +/- 1.14 (80.50%) SKEWNESS = .84

PERCENTILE: 00.1% OF COUNTS IS AT 7.90 MICRONS AND LARGER
PERCENTILE: 01.0% OF COUNTS IS AT 5.39 MICRONS AND LARGER
PERCENTILE: 06.0% OF COUNTS IS AT 3.56 MICRONS AND LARGER
PERCENTILE: 22.0% OF COUNTS IS AT 1.97 MICRONS AND LARGER
PERCENTILE: 50.0% OF COUNTS IS AT .79 MICRONS AND LARGER
PERCENTILE: 78.0% OF COUNTS IS AT .51 MICRONS AND LARGER
PERCENTILE: 94.0% OF COUNTS IS AT .46 MICRONS AND LARGER
PERCENTILE: 99.0% OF COUNTS IS AT .43 MICRONS AND LARGER
PERCENTILE: 99.9% OF COUNTS IS AT .43 MICRONS AND LARGER

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 MAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5656

CLIENT: BATTELLE COLUMBUS LABS 20 DEC 85 :DATA
SAMPLE: DIXON #82 NATURAL GRAPHITE I-9742 :JOB NUMBER

PARTICLE SIZE VS. COUNTS
ENCLOSING
LOW AT 1 .43 8157 HIGH AT 110 18.78 2

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL COUNTS FROM CHANNEL 1 TO 110, AND SKIP: 2

% MAX	SIZE	0 10 20 30 40 50 60 70 80 90 100
99.6	.43	
99.9	.46	
99.4	.53	
97.8	.59	
95.5	.65	
92.4	.72	
88.6	.80	
84.3	.89	
79.4	.99	
74.1	1.10	
68.7	1.22	
63.6	1.35	
57.3	1.50	
52.4	1.66	
51.6	1.84	
48.6	2.04	
48.3	2.27	
44.7	2.52	
42.8	2.79	
40.1	3.10	
38.6	3.44	
27.3	3.81	
20.7	4.23	
15.2	4.70	
7.9	5.21	
5.7	5.78	
3.1	6.41	
1.7	7.12	
.9	7.90	
.5	8.76	
.3	9.72	
.2	10.79	
.1	11.97	
.1	13.28	
.0	14.74	
.0	16.35	
.0	18.14	

% MAX SIZE 0 10 20 30 40 50 60 70 80 90 100

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 20 DEC 85 :DATA
SAMPLE: DIXON KS2 NATURAL GRAPHITE I-9742 :JOB NUMBER

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE 19 DEC
SIZE-NORMALIZED COUNTS DISTRIBUTION
TOTAL = 372481

CHNL	SIZE	COUNTS	%	CHNL	SIZE	COUNTS	%	CHNL	SIZE	COUNTS	%
1	.43	8157	100.00	38	1.55	4488	30.64	75	5.58	545	.36
2	.44	8189	97.81	39	1.60	4365	29.63	76	5.76	470	.71
3	.46	8191	95.61	40	1.66	4292	28.46	77	5.98	404	.58
4	.48	8183	93.41	41	1.72	4264	27.31	78	6.20	338	.48
5	.49	8176	91.22	42	1.78	4265	26.16	79	6.41	258	.38
6	.51	8157	89.02	43	1.84	4231	25.01	80	6.64	204	.32
7	.53	8142	86.83	44	1.91	4126	23.88	81	6.87	179	.26
8	.55	8108	84.64	45	1.97	4046	22.77	82	7.12	136	.21
9	.57	8065	82.47	46	2.04	3981	21.68	83	7.37	115	.18
10	.59	8014	80.30	47	2.12	3905	20.61	84	7.63	94	.15
11	.61	7963	78.15	48	2.19	3929	19.57	85	7.90	77	.12
12	.63	7897	76.01	49	2.27	3953	18.51	86	8.18	62	.10
13	.65	7827	73.89	50	2.35	3873	17.45	87	8.46	51	.08
14	.67	7750	71.79	51	2.43	3794	16.41	88	8.76	38	.07
15	.70	7661	69.71	52	2.52	3658	15.39	89	9.07	32	.06
16	.72	7570	67.65	53	2.60	3601	14.41	90	9.39	26	.05
17	.75	7476	65.62	54	2.70	3539	13.44	91	9.72	21	.04
18	.77	7370	63.62	55	2.79	3507	12.49	92	10.06	17	.04
19	.80	7261	61.64	56	2.89	3469	11.55	93	10.42	17	.03
20	.83	7146	59.67	57	2.99	3384	10.62	94	10.79	13	.03
21	.86	7029	57.77	58	3.10	3284	9.71	95	11.17	13	.03
22	.89	6904	55.88	59	3.21	3113	8.85	96	11.56	11	.02
23	.92	6776	54.03	60	3.32	2937	7.99	97	11.97	11	.02
24	.95	6642	52.21	61	3.44	2767	7.20	98	12.39	9	.02
25	.99	6506	50.43	62	3.56	2637	6.46	99	12.83	8	.01
26	1.02	6369	48.66	63	3.68	2456	5.75	100	13.28	6	.01
27	1.06	6222	46.97	64	3.81	2283	5.09	101	13.75	6	.01
28	1.10	6074	45.30	65	3.95	2128	4.49	102	14.23	6	.01
29	1.13	5927	43.67	66	4.09	1905	3.92	103	14.74	4	.01
30	1.17	5776	42.08	67	4.23	1698	3.41	104	15.26	4	.01
31	1.22	5624	40.53	68	4.38	1566	2.95	105	15.79	4	.00
32	1.26	5471	39.02	69	4.54	1361	2.56	106	16.35	4	.00
33	1.30	5316	37.55	70	4.70	1245	2.16	107	16.93	2	.00
34	1.35	5161	36.12	71	4.86	1175	1.83	108	17.52	2	.00
35	1.40	5005	34.74	72	5.03	975	1.51	109	18.14	2	.00
36	1.45	4827	33.39	73	5.21	811	1.25	110	18.78	2	.00
37	1.50	4690	32.10	74	5.39	658	1.03				

DISPLAY AREA: 4

PARTICLE SIZE ANALYSIS BY [illegible]
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET – ELMHURST, ILL. [illegible]
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS   20 DEC 85 :DATA
SAMPLE: DIXON KS2 NATURAL GRAPHITE   I-9742 :JOB NUMBER

PARTICLE SIZE VS. VOLUME
ENCLOSING
LOW AT 1 .43 626 HIGH AT 121 27.50 1447

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL VOLUME FROM CHANNEL 1 TO 121, AND SKIP: 2

% MAX	SIZE
.5	.43
.7	.48
.9	.53
1.2	.59
1.6	.65
2.1	.72
2.[illegible]	.80
3.6	.89
4.[illegible]	.97
[illegible]	1.10
7.4	1.22
9.[illegible]	1.35
11.6	1.50
14.[illegible]	1.65
[illegible]	1.84
24.7	2.04
34.1	2.27
45.2	2.52
56.3	2.79
72.0	3.10
83.9	3.44
91.5	3.81
94.9	4.23
95.1	4.70
84.6	5.21
[illegible]	5.78
50.3	6.41
36.1	7.12
23.1	7.90
19.1	8.76
14.1	9.72
[illegible]	10.79
[illegible]	11.97
[illegible].6	13.28
10.1	14.74
9.4	16.35
6.[illegible]	18.14
4.1	20.13
3.[illegible]	22.34
2.7	24.78
1.1	27.50

% MAX SIZE 0 10 20 30 40 50 60 70 80 90 100

Appendix [illegible]

PARTICLE SIZE ANALYSIS BY [illegible]
PARTICLE DATA LABORATORIES, LTD.
[illegible]
TELEPHONE: (312) 832-[illegible]

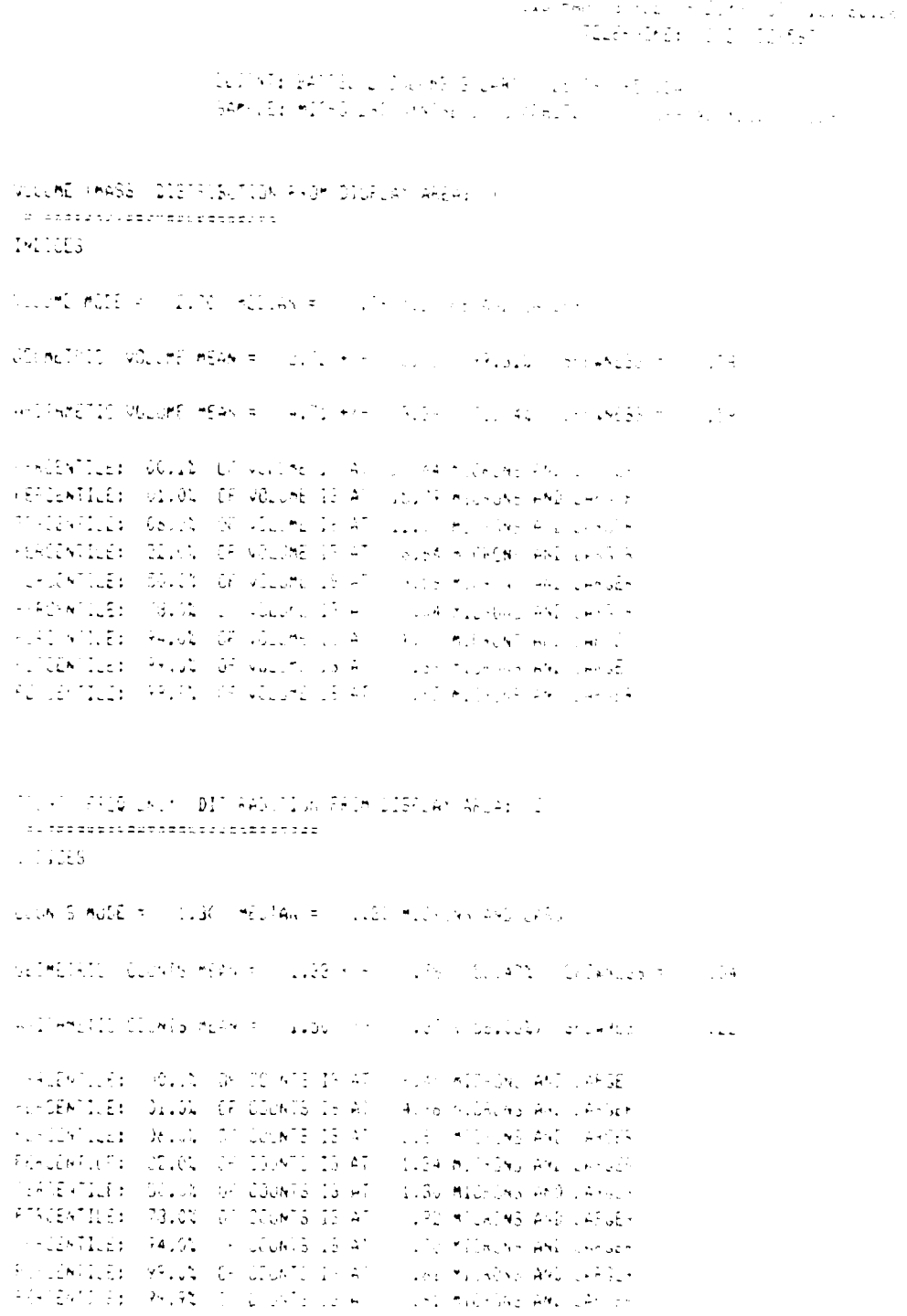
CLIENT: [illegible] CHELLE COLUMBUS LABS [illegible] IIC 85 DATA
SAMPLE: DIXON [illegible] NATURAL [illegible] [illegible] [illegible]

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE 19 DEC
SIZE-NORMALIZED VOLUME DISTRIBUTION
TOTAL = 4173597

CHNL	SIZE	VOLUME	%	CHNL	SIZE	VOLUME	%	CHNL	SIZE	VOLUME	%
1	.43	626	100.00	42	1.78	23898	93.70	83	7.37	48741	13.93
2	.44	697	99.99	43	1.84	25576	93.10	84	7.63	40495	12.78
3	.46	774	99.97	44	1.91	27857	92.52	85	7.90	36636	11.91
4	.48	856	99.95	45	1.97	30099	91.80	86	8.18	3.997	11.13
5	.49	951	99.93	46	2.04	32352	91.14	87	6.46	29865	10.24
6	.51	1053	99.91	47	2.12	36501	90.36	88	8.76	25056	9.53
7	.53	1166	99.88	48	2.19	39945	89.49	89	9.07	23151	8.93
8	.55	1286	99.85	49	2.27	44641	88.58	90	9.39	20400	8.47
9	.57	1421	99.82	50	2.35	48469	87.49	91	9.72	18442	7.85
10	.59	1567	99.79	51	2.43	52610	86.30	92	10.06	17038	7.44
11	.61	1728	99.75	52	2.52	56850	85.04	93	10.42	17604	7.08
12	.63	1901	99.71	53	2.60	63180	83.66	94	10.79	16826	6.81
13	.65	2091	99.66	54	2.70	67455	82.17	95	11.17	17655	6.21
14	.67	2297	99.61	55	2.79	73813	80.65	96	11.56	16718	5.79
15	.70	2520	99.56	56	2.89	75160	78.78	97	11.97	17737	5.59
16	.72	2762	99.50	57	2.99	85496	76.98	98	12.39	16877	4.96
17	.75	3027	99.43	58	3.10	94432	74.93	99	12.82	15634	4.57
18	.77	3311	99.36	59	3.21	99482	72.87	100	13.26	15063	4.41
19	.80	3620	99.28	60	3.32	103972	70.28	101	13.75	15662	3.86
20	.83	3954	99.19	61	3.44	108076	67.79	102	14.23	15099	3.49
21	.86	4313	99.10	62	3.56	114932	65.19	103	14.74	13184	3.13
22	.89	4707	99.00	63	3.68	114663	62.43	104	15.26	13892	2.81
23	.92	5120	98.88	64	3.81	119940	59.69	105	15.79	12670	2.49
24	.95	5568	98.76	65	3.95	126568	56.91	106	16.35	12359	2.24
25	.99	6052	98.63	66	4.09	125859	53.76	107	16.92	10481	1.99
26	1.02	6579	98.48	67	4.23	124437	50.76	108	17.52	9391	1.64
27	1.06	7125	98.32	68	4.38	127033	47.79	109	18.14	8179	1.42
28	1.10	7719	98.15	69	4.54	131071	44.79	110	18.78	7947	1.32
29	1.13	8356	97.97	70	4.70	124606	41.69	111	19.44	6415	1.03
30	1.17	9037	97.77	71	4.86	130561	38.68	112	20.13	5428	.93
31	1.22	9763	97.55	72	5.03	133219	35.48	113	20.84	4814	.79
32	1.26	10536	97.32	73	5.21	110945	32.08	114	21.57	4[illegible]	.68
33	1.30	11361	97.07	74	5.39	99411	29.04	115	22.34	3464	.62
34	1.35	12237	96.79	75	5.58	91799	27.04	116	23.12	4[illegible]	.[illegible]
35	1.40	13167	96.50	76	5.78	87761	25.34	117	23.94	[illegible]	.55
36	1.45	14375	96.18	77	5.98	[illegible]	23.24	118	[illegible]	[illegible]	[illegible]
37	1.50	15184	95.84	78	6.19	[illegible]	[illegible]	119	[illegible]	[illegible]	.43
38	1.55	16128	95.48	79	6.41	65944	19.04	[illegible]	[illegible]	[illegible]	[illegible]
39	1.60	17943	95.09	80	6.64	[illegible]	[illegible]	121	[illegible]	44	.34
40	1.66	18489	94.66	81	6.87	58311	16.84				
41	1.72	21033	94.20	82	7.12	47302	[illegible]				



Appendix A

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 16 DEC 85 :DATA
SAMPLE: MICRO 260 SYNTHETIC GRAPHITE I-97-2 :LOG NUMBER

PARTICLE SIZE VS. COUNTS
ENCLOSING
LOW AT 1 .61 1675 HIGH AT 96 16.35 1

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL COUNTS FROM CHANNEL 1 TO 96, AND SKIP: 2

% MAX	SIZE
40.9	.61
52.2	.67
63.9	.75
75.3	.83
85.1	.92
92.4	1.02
96.4	1.13
99.2	1.26
98.6	1.40
91.7	1.55
77.2	1.72
63.9	1.91
51.6	2.12
39.5	2.35
30.9	2.60
21.9	2.89
16.7	3.21
11.2	3.56
8.3	3.95
6.1	4.38
4.3	4.86
3.0	5.39
1.8	5.98
1.3	6.64
.9	7.37
.7	8.18
.4	9.07
.3	10.06
.2	11.17
.1	12.39
.0	13.75
.0	15.26

% MAX SIZE 0 10 20 30 40 50 60 70 80 90 100

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 26 DEC 85 :DATA
SAMPLE: MICRO 260 SYNTHETIC GRAPHITE I-9742 :JOB NUMBER

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE 26 DEC
SIZE-NORMALIZED COUNTS DISTRIBUTION
TOTAL = 138131

CHNL	SIZE	COUNTS	% >	CHNL	SIZE	COUNTS	% >	CHNL	SIZE	COUNTS	%
1	.61	1675	100.00	33	1.84	2876	23.51	65	5.58	111	.81
2	.63	1826	98.79	34	1.91	2818	21.43	66	5.78	88	.63
3	.65	1981	97.47	35	1.97	2455	19.54	67	5.98	75	.47
4	.67	2140	96.03	36	2.04	2252	17.76	68	6.20	73	.41
5	.70	2299	94.43	37	2.12	2112	16.13	69	6.41	63	.36
6	.72	2461	92.62	38	2.19	1951	14.60	70	6.64	55	.31
7	.75	2619	91.04	39	2.27	1777	13.19	71	6.87	53	.27
8	.77	2778	89.14	40	2.35	1617	11.90	72	7.12	44	.25
9	.80	2933	87.13	41	2.43	1520	10.73	73	7.37	38	.20
10	.83	3083	85.01	42	2.52	1376	9.63	74	7.63	33	.18
11	.86	3227	82.77	43	2.60	1265	8.63	75	7.90	27	.15
12	.89	3360	80.44	44	2.70	1152	7.72	76	8.18	27	.13
13	.92	3485	78.00	45	2.79	1007	6.86	77	8.46	22	.11
14	.95	3599	75.48	46	2.89	897	6.13	78	8.76	20	.10
15	.99	3700	72.88	47	2.99	854	5.48	79	9.07	16	.08
16	1.02	3785	70.20	48	3.10	713	4.86	80	9.39	14	.07
17	1.06	3866	67.46	49	3.21	665	4.35	81	9.72	13	.06
18	1.10	3932	64.67	50	3.32	580	3.85	82	10.06	12	.05
19	1.13	3980	61.83	51	3.44	519	3.43	83	10.42	10	.04
20	1.17	3991	58.97	52	3.56	459	3.06	84	10.79	9	.03
21	1.21	4020	56.09	53	3.68	429	2.72	85	11.17	7	.03
22	1.26	4064	53.17	54	3.81	364	2.42	86	11.56	6	.02
23	1.30	4095	50.23	55	3.95	342	2.15	87	11.97	5	.02
24	1.35	4072	47.27	56	4.09	295	1.90	88	12.39	4	.01
25	1.40	4040	44.32	57	4.23	251	1.68	89	12.83	3	.01
26	1.45	3970	41.40	58	4.38	249	1.50	90	13.28	3	.01
27	1.50	3899	38.52	59	4.54	209	1.33	91	13.75	2	.01
28	1.55	3794	35.69	60	4.70	184	1.17	92	14.23	2	.01
29	1.60	3514	32.98	61	4.86	177	1.04	93	14.74	2	.00
30	1.66	3373	30.43	62	5.03	165	.91	94	15.26	1	.00
31	1.72	3162	27.99	63	5.21	130	.79	95	15.79	1	.00
32	1.78	3024	25.70	64	5.39	122	.70	96	16.35	1	.00

DISPLAY AREA: 4

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
[illegible] MAIN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5588

CLIENT: BATTELLE COLUMBUS LABS 26 DEC 85 :DATA
SAMPLE: MICRO 260 SYNTHETIC GRAPHITE 1-9742 :JOB NUMBER

PARTICLE SIZE VS. VOLUME
ENCLOSING
LOW AT 1 .61 531 HIGH AT 103 20.84 396

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL VOLUME FROM CHANNEL 1 TO 103, AND SKIP: 2

% MAX	SIZE	0 10 20 30 40 50 60 70 80 90 100
1.6	.61	
2.8	.67	
4.7	.75	
7.6	.83	
11.7	.92	
17.4	1.02	
24.8	1.13	
35.4	1.26	
45.6	1.40	
60.2	1.55	
69.2	1.72	
78.3	1.91	
89.0	2.12	
90.3	2.35	
96.5	2.60	
93.4	2.89	
97.4	3.21	
88.3	3.56	
90.8	3.95	
90.5	4.38	
87.4	4.86	
83.0	5.39	
69.1	5.98	
70.4	6.64	
66.5	7.37	
62.4	8.18	
56.3	9.07	
50.1	10.06	
42.2	11.17	
33.7	12.39	
24.0	13.75	
18.2	15.26	
8.4	16.93	
4.5	18.78	
1.2	20.84	
% MAX	SIZE	0 10 20 30 40 50 60 70 80 90 100

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 MANN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5655

CLIENT: BATTELLE COLUMBUS LABS 26 DEC 85 :DATA
SAMPLE: MICRO 260 SYNTHETIC GRAPHITE I-9742 :JOB NUMBER

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE 26 DEC
SIZE-NORMALIZED VOLUME DISTRIBUTION
TOTAL = 1738320

CHNL	SIZE	VOLUME	% >	CHNL	SIZE	VOLUME	% >	CHNL	SIZE	VOLUME	% >
1	.61	531	100.00	36	2.04	27172	79.56	71	6.87	24235	21.96
2	.63	643	99.97	37	2.12	29169	78.00	72	7.12	22061	20.57
3	.65	773	99.93	38	2.19	30156	76.32	73	7.37	21601	19.30
4	.67	927	99.89	39	2.27	29287	74.58	74	7.63	20816	18.05
5	.70	1104	99.83	40	2.35	29574	72.90	75	7.90	18398	16.85
6	.72	1312	99.77	41	2.43	30661	71.20	76	8.18	20443	15.79
7	.75	1550	99.70	42	2.52	30967	69.42	77	8.46	18984	14.61
8	.77	1824	99.61	43	2.60	31610	67.64	78	8.76	18976	13.52
9	.80	2137	99.50	44	2.70	32767	65.82	79	9.07	19100	12.43
10	.83	2492	99.36	45	2.79	30982	63.94	80	9.39	16635	11.33
11	.86	2894	99.24	46	2.89	30606	62.16	81	9.72	17015	10.37
12	.89	3344	99.07	47	2.99	32328	60.40	82	10.06	16424	9.40
13	.92	3848	98.89	48	3.10	29968	58.54	83	10.42	15022	8.45
14	.95	4409	98.66	49	3.21	31914	56.81	84	10.79	14284	7.59
15	.99	5027	98.40	50	3.32	30024	54.98	85	11.17	13827	6.77
16	1.02	5709	98.11	51	3.44	29746	53.25	86	11.56	13458	5.97
17	1.06	454	97.78	52	3.56	29083	51.54	87	11.97	12382	5.20
18	1.10	7263	97.41	53	3.68	30331	49.80	88	12.39	11031	4.48
19	1.13	8128	96.99	54	3.81	28927	48.12	89	12.83	10.43	3.85
20	1.17	9121	96.53	55	3.95	29744	46.45	90	13.28	9110	3.27
21	1.22	10309	96....	56	4.09	29398	44. -	91	13.75	7867	2.74
22	1.26	11609	95.40	57	4.23	26882	43.09	92	14.23	7793	2.29
23	1.30	12467	94.73	58	4.38	29639	41.54	93	14.74	7325	1.84
24	1.35	14247	94.01	59	4.54	27461	39.84	94	15.26	5963	1.42
25	1.40	16006	93.19	60	4.70	26[illegible]	38.26	95	15.[illegible]9	4493	1.08
26	1.45	16961	92.22	61	4.86	28621	36.71	96	16.35	3866	.82
27	1.50	18456	91.30	62	5.03	29267	35.06	97	16.93	2751	.59
28	1.55	19715	90.23	63	5.21	25668	33.37	98	17.52	2511	.44
29	1.60	20475	89.10	64	5.39	27206	31.89	99	18.14	1691	.29
30	1.66	21807	87.92	65	5.58	27148	30.32	100	18.78	1470	.19
31	1.72	22685	86.67	66	5.78	24211	28.76	101	19.44	859	.11
32	1.78	24067	85.36	67	5.98	22640	27.37	102	20.13	664	.06
33	1.84	23003	83.98	68	6.20	24718	26.06	103	20.84	396	.02
34	1.91	25650	82.65	69	6.41	23499	24.64				
35	1.97	28062	81.17	70	6.64	23074	23.29				

#

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 19 DEC 85 :DATA
SAMPLE: CARBON BLACK (PRINTEX L) I-9742 :JOB NUMBER

VOLUME (MASS) DISTRIBUTION FROM DISPLAY AREA: 4
==========
INDICES

VOLUME MODE = 65.40 MEDIAN = 40.61 MICRONS AND LARGER

GEOMETRIC VOLUME MEAN = 26.09 +/- 52.84 (202.51%) SKEWNESS = -.74

ARITHMETIC VOLUME MEAN = 39.40 +/- 26.25 (66.62%) SKEWNESS = -.99

PERCENTILE: 00.1% OF VOLUME IS AT 96.59 MICRONS AND LARGER
PERCENTILE: 01.0% OF VOLUME IS AT 88.57 MICRONS AND LARGER
PERCENTILE: 06.0% OF VOLUME IS AT 77.78 MICRONS AND LARGER
PERCENTILE: 22.0% OF VOLUME IS AT 65.40 MICRONS AND LARGER
PERCENTILE: 50.0% OF VOLUME IS AT 40.61 MICRONS AND LARGER
PERCENTILE: 78.0% OF VOLUME IS AT 10.60 MICRONS AND LARGER
PERCENTILE: 94.0% OF VOLUME IS AT 2.89 MICRONS AND LARGER
PERCENTILE: 99.0% OF VOLUME IS AT 1.33 MICRONS AND LARGER
PERCENTILE: 99.9% OF VOLUME IS AT 1.02 MICRONS AND LARGER

COUNT (FREQUENCY) DISTRIBUTION FROM DISPLAY AREA: 5
==========
INDICES

COUNTS MODE = .98 MEDIAN = 1.38 MICRONS AND LARGER

GEOMETRIC COUNTS MEAN = 1.57 +/- .86 (54.49%) SKEWNESS = .69

ARITHMETIC COUNTS MEAN = 1.78 +/- 1.51 (84.63%) SKEWNESS = .53

PERCENTILE: 00.1% OF COUNTS IS AT 17.07 MICRONS AND LARGER
PERCENTILE: 01.0% OF COUNTS IS AT 6.87 MICRONS AND LARGER
PERCENTILE: 06.0% OF COUNTS IS AT 3.44 MICRONS AND LARGER
PERCENTILE: 22.0% OF COUNTS IS AT 2.04 MICRONS AND LARGER
PERCENTILE: 50.0% OF COUNTS IS AT 1.38 MICRONS AND LARGER
PERCENTILE: 78.0% OF COUNTS IS AT 1.11 MICRONS AND LARGER
PERCENTILE: 94.0% OF COUNTS IS AT .98 MICRONS AND LARGER
PERCENTILE: 99.0% OF COUNTS IS AT .98 MICRONS AND LARGER
PERCENTILE: 99.9% OF COUNTS IS AT .98 MICRONS AND LARGER

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 19 DEC 85 :DATA
SAMPLE: CARBON BLACK (PRINTEX L) I-9742 :JOB NUMBER

PARTICLE SIZE VS. COUNTS
ENCLOSING
LOW AT 21 .98 131071 HIGH AT 126 92.49 1

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL COUNTS FROM CHANNEL 21 TO 126, AND SKIP: 2

% MAX	SIZE
.0	.90
99.6	1.02
92.1	1.16
79.4	1.33
65.1	1.51
50.5	1.72
37.9	1.96
28.3	2.23
20.5	2.54
15.2	2.89
10.7	3.29
7.8	3.75
5.9	4.27
4.6	4.86
3.5	5.54
2.6	6.30
1.8	7.18
1.2	8.18
.9	9.31
.6	10.60
.4	12.07
.3	13.75
.2	15.66
.1	17.83
.1	20.30
.1	23.12
.1	26.33
.0	29.99
.0	34.15
.0	38.89
.0	44.29
.0	50.43
.0	57.43
.0	65.40
.0	74.48
.0	84.82

Scale: 0 10 20 30 40 50 60 70 80 90 100

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 19 DEC 85 :DATA
SAMPLE: CARBON BLACK (PRINTEX L) I-9742 :JOB NUMBER

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE 17 DEC
SIZE-NORMALIZED COUNTS DISTRIBUTION
TOTAL = 208569

CHNL	SIZE	COUNTS	% >	CHNL	SIZE	COUNTS	% >	CHNL	SIZE	COUNTS	% >
21	.98	131071	100.00	57	4.65	6622	3.12	93	22.14	101	.06
22	1.02	130594	93.72	58	4.86	5973	2.81	94	23.12	90	.05
23	1.07	127593	87.45	59	5.08	5560	2.52	95	24.15	82	.05
24	1.11	124421	81.34	60	5.30	5102	2.25	96	25.22	75	.05
25	1.16	120761	75.37	61	5.54	4567	2.01	97	26.33	68	.04
26	1.22	115424	69.58	62	5.78	4021	1.79	98	27.50	64	.04
27	1.27	110413	64.05	63	6.04	3737	1.60	99	28.72	60	.04
28	1.33	104108	58.75	64	6.30	3377	1.42	100	29.99	54	.03
29	1.38	98335	53.76	65	6.58	2997	1.26	101	31.31	50	.03
30	1.45	92443	49.05	66	6.87	2604	1.11	102	32.70	48	.03
31	1.51	85281	44.61	67	7.18	2420	.99	103	34.15	44	.03
32	1.58	78552	40.53	68	7.50	2100	.87	104	35.66	41	.02
33	1.65	72335	36.76	69	7.83	1803	.77	105	37.24	39	.02
34	1.72	66251	33.29	70	8.18	1625	.69	106	38.89	38	.02
35	1.79	61255	30.12	71	8.54	1417	.61	107	40.61	36	.02
36	1.87	55052	27.18	72	8.92	1251	.54	108	42.41	35	.02
37	1.96	49618	24.54	73	9.31	1140	.48	109	44.29	34	.02
38	2.04	46072	22.16	74	9.72	980	.42	110	46.25	32	.01
39	2.13	41773	19.92	75	10.15	877	.38	111	48.29	30	.01
40	2.23	37092	17.91	76	10.60	748	.34	112	50.43	29	.01
41	2.33	33447	16.14	77	11.07	668	.30	113	52.66	27	.01
42	2.43	30390	14.54	78	11.56	600	.27	114	55.00	25	.01
43	2.54	26806	13.08	79	12.07	530	.24	115	57.43	23	.01
44	2.65	24919	11.79	80	12.61	478	.21	116	59.97	21	.01
45	2.77	22263	10.60	81	13.17	406	.19	117	62.63	19	.00
46	2.89	19929	9.53	82	13.75	356	.17	118	65.40	17	.00
47	3.02	17506	8.58	83	14.36	324	.15	119	68.30	15	.00
48	3.15	15697	7.74	84	14.99	288	.14	120	71.32	13	.00
49	3.29	14039	6.98	85	15.66	253	.12	121	74.48	11	.00
50	3.44	12831	6.31	86	16.35	217	.11	122	77.78	9	.00
51	3.59	10895	5.70	87	17.07	198	.10	123	81.22	5	.00
52	3.75	10277	5.17	88	17.83	169	.09	124	84.82	3	.00
53	3.91	9011	4.68	89	18.62	151	.08	125	88.57	2	.00
54	4.09	8678	4.25	90	19.44	134	.08	126	92.49	1	.00
55	4.27	7712	3.83	91	20.30	122	.07				
56	4.46	7049	3.46	92	21.20	110	.06				

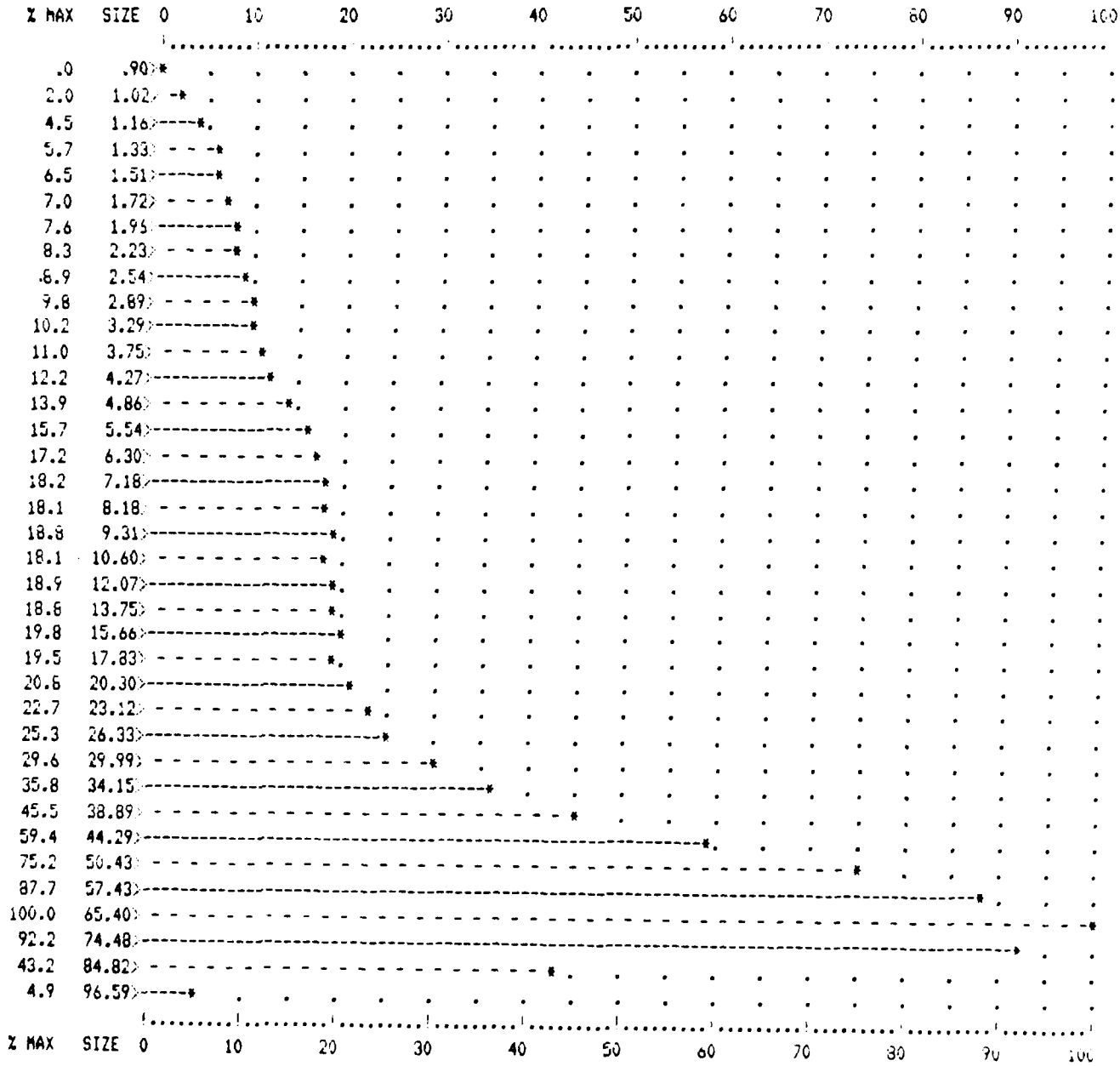
DISPLAY AREA: 4

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5656

CLIENT: BATTELLE COLUMBUS LABS 19 DEC 85 :DATA
SAMPLE: CARBON BLACK (PRINTEX L) I-9742 :JOB NUMBER

PARTICLE SIZE VS. VOLUME
ENCLOSING
LOW AT 21 .98 113014 HIGH AT 128 100.86 83806

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL VOLUME FROM CHANNEL 21 TO 128, AND SKIP: 2



PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 19 DEC 85 :DATA
SAMPLE: CARBON BLACK (PRINTEX L) I-9742 :JOB NUMBER

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE 17 DEC
SIZE-NORMALIZED VOLUME DISTRIBUTION
TOTAL = 2.34557E 8

CHNL	SIZE	VOLUME	% >	CHNL	SIZE	VOLUME	% >	CHNL	SIZE	VOLUME	% >
21	.98	113014	100.00	57	4.65	1139050	89.50	93	22.14	1868157	66.55
22	1.02	167281	99.95	58	4.86	1169740	89.41	94	23.12	1900987	65.76
23	1.07	265786	99.88	59	5.08	1240080	88.91	95	24.15	1980900	64.95
24	1.11	331249	99.77	60	5.30	1295868	88.38	96	25.22	2037185	64.10
25	1.16	373567	99.63	61	5.54	1321036	87.83	97	26.33	2123167	63.23
26	1.22	419057	99.47	62	5.78	1324451	87.27	98	27.50	2254622	62.33
27	1.27	456502	99.29	63	6.04	1401986	86.70	99	28.72	2389239	61.37
28	1.33	480424	99.09	64	6.30	1442844	86.11	100	29.99	2486326	60.43
29	1.38	506461	98.89	65	6.58	1457764	85.49	101	31.31	2624794	59.29
30	1.45	536796	98.67	66	6.87	1442729	84.87	102	32.70	2858182	58.17
31	1.51	541702	98.44	67	7.18	1526626	84.25	103	34.15	3001912	56.95
32	1.58	568051	98.21	68	7.50	1508673	83.60	104	35.66	3222686	55.67
33	1.65	589905	97.97	69	7.83	1474965	82.96	105	37.24	3424992	54.30
34	1.72	590993	97.72	70	8.18	1514206	82.33	106	38.89	3814739	52.64
35	1.79	603746	97.47	71	8.54	1502799	81.68	107	40.61	4043601	51.21
36	1.87	617852	97.21	72	8.92	1511244	81.04	108	42.41	4486481	49.49
37	1.96	634041	96.95	73	9.31	1579701	80.40	109	44.29	4979568	47.57
38	2.04	680013	96.68	74	9.72	1536585	79.73	110	46.25	5555709	45.45
39	2.13	692526	96.39	75	10.15	1565450	79.07	111	48.29	5899403	43.08
40	2.23	700254	96.09	76	10.60	1521683	78.40	112	50.43	6309453	40.57
41	2.33	719096	95.79	77	11.07	1547694	77.76	113	52.66	6759931	37.88
42	2.43	744056	95.49	78	11.56	1582154	77.10	114	55.00	7014827	34.79
43	2.54	747445	95.17	79	12.07	1588766	76.42	115	57.43	7355336	32.00
44	2.65	791191	94.85	80	12.61	1632549	75.74	116	59.97	7658623	28.67
45	2.77	805003	94.51	81	13.17	1582507	75.05	117	62.63	8071296	25.60
46	2.89	820605	94.17	82	13.75	1577137	74.37	118	65.40	8388607	22.16
47	3.02	820867	93.82	83	14.36	1632608	73.70	119	68.30	8349041	18.59
48	3.15	838198	93.47	84	14.99	1655698	73.00	120	71.32	8210802	15.03
49	3.29	853685	93.11	85	15.66	1657609	72.30	121	74.48	7735605	11.53
50	3.44	888501	92.75	86	16.35	1616436	71.5.	122	77.7s	6562935	8.23
51	3.59	859063	92.37	87	17.07	1687021	70.90	123	81.22	5080311	5.43
52	3.75	922883	92.00	88	17.83	1634678	70.18	124	84.82	3627739	3.26
53	3.91	921172	91.61	89	18.62	1655777	69.49	125	88.57	2366650	1.72
54	4.09	1010719	91.22	90	19.44	1687978	68.78	126	92.49	1162315	.71
55	4.27	1022826	90.79	91	20.30	1741192	68.06	127	96.59	412395	.21
56	4.46	1064590	90.35	92	21.20	1795965	67.32	128	100.86	83806	.04

#

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 MAIN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5e58

CLIENT: BATTELLE COLUMBUS LABS 20 DEC 85 :DATA
SAMPLE: ALUMINUM DUST I-9742 :JOB NUMBER

VOLUME (MASS) DISTRIBUTION FROM DISPLAY AREA: 4
==========
INDICES

VOLUME MODE = 9.07 MEDIAN = 6.41 MICRONS AND LARGER

GEOMETRIC VOLUME MEAN = 5.94 +/- 8.30 (139.70%) SKEWNESS = -.38

ARITHMETIC VOLUME MEAN = 8.26 +/- 6.34 (76.75%) SKEWNESS = -.13

PERCENTILE: 00.1% OF VOLUME IS AT 31.59 MICRONS AND LARGER
PERCENTILE: 01.0% OF VOLUME IS AT 26.5e MICRONS AND LARGER
PERCENTILE: 06.0% OF VOLUME IS AT 20.1e MICRONS AND LARGER
PERCENTILE: 22.0% OF VOLUME IS AT 12.83 MICRONS AND LARGER
PERCENTILE: 50.0% OF VOLUME IS AT 6.41 MICRONS AND LARGER
PERCENTILE: 78.0% OF VOLUME IS AT 2.99 MICRONS AND LARGER
PERCENTILE: 94.0% OF VOLUME IS AT 1.35 MICRONS AND LARGER
PERCENTILE: 99.0% OF VOLUME IS AT .65 MICRONS AND LARGER
PERCENTILE: 99.9% OF VOLUME IS AT .4e MICRONS AND LARGER

COUNT (FREQUENCY) DISTRABUTION FROM DISPLAY AREA: 5.
==========
INDICES

COUNTS MODE = .43 MEDIAN = .70 MICRONS AND LARGER

GEOMETRIC COUNTS MEAN = .81 +/- .59 (72.69%) SKEWNESS = .65

ARITHMETIC COUNTS MEAN = .98 +/- .86 (87.57%) SKEWNESS = .64

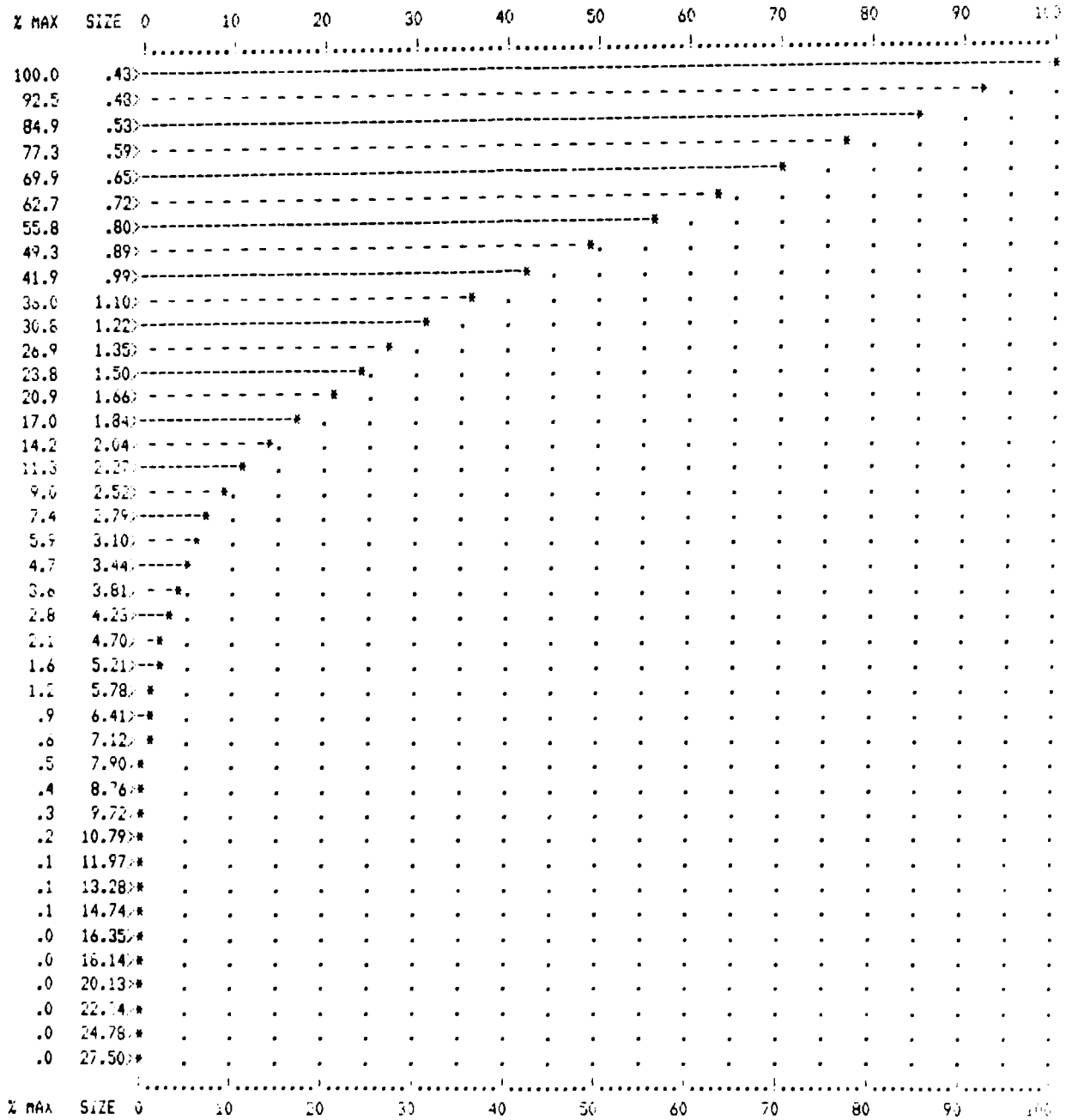
PERCENTILE: 00.1% OF COUNTS IS AT 9.39 MICRONS AND LARGER
PERCENTILE: 01.0% OF COUNTS IS AT 4.38 MICRONS AND LARGER
PERCENTILE: 06.0% OF COUNTS IS AT 2.19 MICRONS AND LARGER
PERCENTILE: 22.0% OF COUNTS IS AT 1.17 MICRONS AND LARGER
PERCENTILE: 50.0% OF COUNTS IS AT .70 MICRONS AND LARGER
PERCENTILE: 78.0% OF COUNTS IS AT .51 MICRONS AND LARGER
PERCENTILE: 94.0% OF COUNTS IS AT .44 MICRONS AND LARGER
PERCENTILE: 99.0% OF COUNTS IS AT .43 MICRONS AND LARGER
PERCENTILE: 99.9% OF COUNTS IS AT .43 MICRONS AND LARGER

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 MAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 20 DEC 85 :DATA
SAMPLE: ALUMINUM DUST I-9742 :JOB NUMBER

PARTICLE SIZE VS. COUNTS
ENCLOSING
LOW AT 1 .43 65535 HIGH AT 123 29.47 1

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL COUNTS FROM CHANNEL 1 TO 123, AND SKIP: 2



PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 20 DEC 85 :DATA
SAMPLE: ALUMINUM DUST I-9742 :JOB NUMBER

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE 20 DEC
SIZE-NORMALIZED COUNTS DISTRIBUTION
TOTAL = 1617360

CHNL	SIZE	COUNTS	% >	CHNL	SIZE	COUNTS	% >	CHNL	SIZE	COUNTS	% >
1	.43	65535	100.00	42	1.78	1736	10.23	83	7.37	399	.25
2	.44	63903	95.95	43	1.84	11118	9.51	84	7.63	353	.21
3	.46	62259	92.00	44	1.91	10579	8.82	85	7.90	299	.18
4	.48	60613	88.15	45	1.97	10063	8.17	86	8.18	299	.17
5	.49	58959	84.40	46	2.04	9280	7.55	87	8.46	258	.15
6	.51	57275	80.75	47	2.12	8465	6.97	88	8.76	238	.13
7	.53	55621	77.21	48	2.19	8026	6.45	89	9.07	225	.12
8	.55	53969	73.77	49	2.27	7409	5.95	90	9.39	194	.10
9	.57	52317	70.44	50	2.35	6866	5.49	91	9.72	169	.09
10	.59	50673	67.20	51	2.43	6269	5.07	92	10.06	147	.08
11	.61	49039	64.07	52	2.52	5900	4.68	93	10.42	130	.07
12	.63	47415	61.04	53	2.60	5840	4.32	94	10.79	120	.06
13	.65	45805	58.11	54	2.70	5439	3.96	95	11.17	115	.06
14	.67	44213	55.27	55	2.79	4852	3.62	96	11.56	85	.05
15	.70	42637	52.54	56	2.89	4554	3.32	97	11.97	87	.04
16	.72	41086	49.90	57	2.99	4268	3.04	98	12.39	72	.04
17	.75	39556	47.36	58	3.10	3852	2.77	99	12.83	63	.03
18	.77	38052	44.92	59	3.21	3502	2.54	100	13.28	58	.03
19	.80	36572	42.57	60	3.32	3124	2.32	101	13.75	52	.03
20	.83	35121	40.30	61	3.44	3083	2.13	102	14.23	46	.02
21	.86	33698	38.13	62	3.56	2898	1.94	103	14.74	47	.02
22	.89	32306	36.05	63	3.68	2464	1.76	104	15.26	41	.02
23	.92	31010	34.05	64	3.81	2357	1.60	105	15.79	33	.01
24	.95	29034	32.14	65	3.95	2093	1.46	106	16.35	30	.01
25	.99	27483	30.34	66	4.09	1954	1.33	107	16.93	26	.01
26	1.02	26404	28.64	67	4.23	1811	1.21	108	17.52	25	.01
27	1.06	24670	27.01	68	4.38	1644	1.10	109	18.14	20	.01
28	1.10	23610	25.49	69	4.54	1547	.99	110	18.78	17	.01
29	1.13	22157	24.03	70	4.70	1404	.90	111	19.44	15	.00
30	1.17	21095	22.66	71	4.86	1255	.81	112	20.13	13	.00
31	1.22	20168	21.35	72	5.03	1166	.73	113	20.84	10	.00
32	1.26	19127	20.11	73	5.21	1066	.66	114	21.57	9	.00
33	1.30	18482	18.92	74	5.39	991	.60	115	22.34	7	.00
34	1.35	17615	17.78	75	5.58	886	.53	116	23.12	6	.00
35	1.40	16721	16.69	76	5.78	777	.48	117	23.94	4	.00
36	1.45	16224	15.66	77	5.98	702	.43	118	24.78	4	.00
37	1.50	15596	14.65	78	6.20	621	.39	119	25.66	2	.00
38	1.55	15175	13.69	79	6.41	563	.35	120	26.56	2	.00
39	1.60	14436	12.75	80	6.64	523	.32	121	27.50	1	.00
40	1.66	13695	11.86	81	6.87	439	.28	122	28.47	1	.00
41	1.72	12583	11.01	82	7.12	414	.26	123	29.47	1	.00

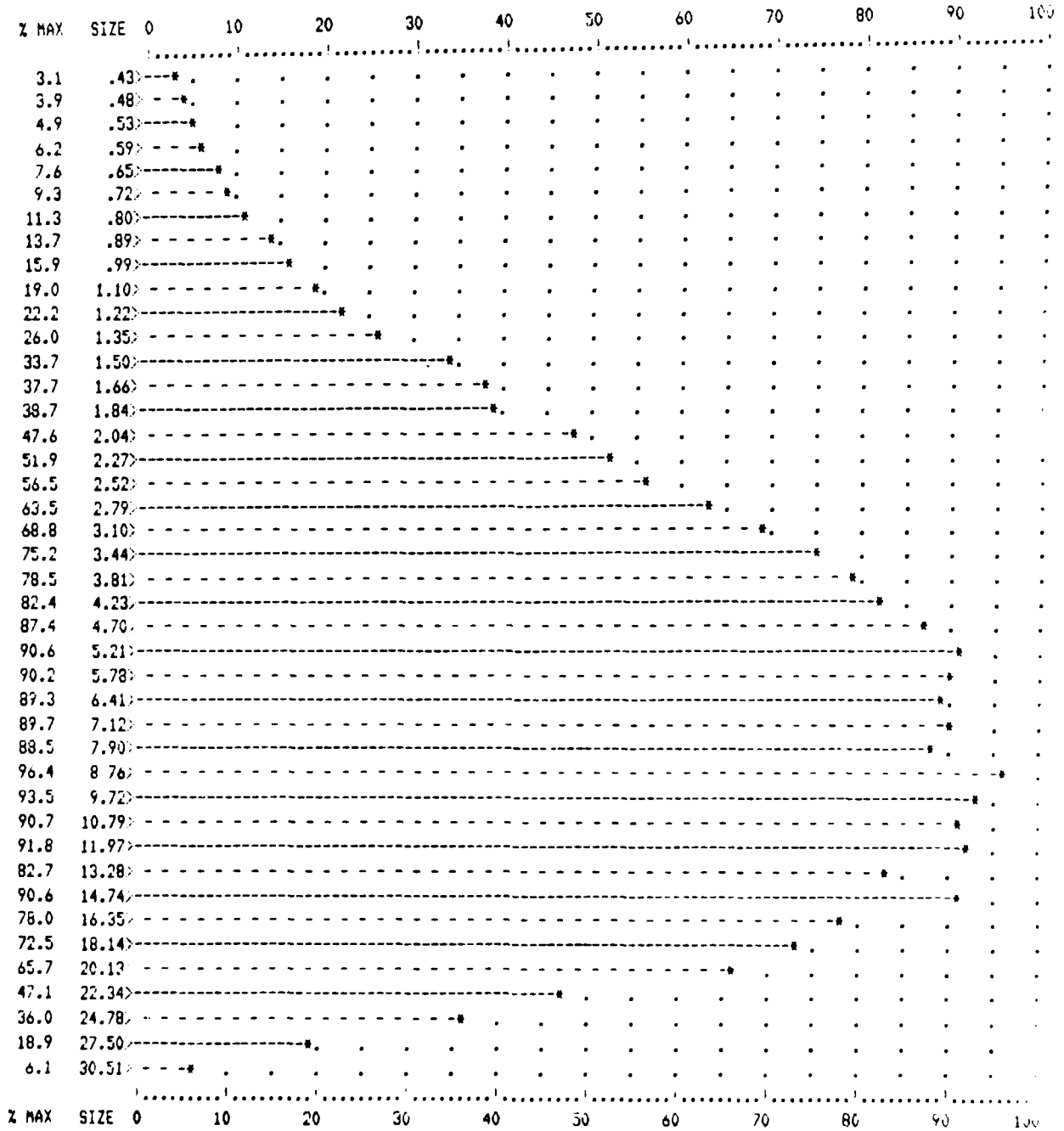
DISPLAY AREA: 4

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5656

CLIENT: BATTELLE COLUMBUS LABS 20 DEC 85 :DATA
SAMPLE: ALUMINUM DUST I-9742 :JOB NUMBER

PARTICLE SIZE VS. VOLUME
ENCLOSING
LOW AT 1 .43 8189 HIGH AT 126 32.70 7449

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL VOLUME FROM CHANNEL 1 TO 126, AND SKIP: 2



PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 MAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5656

CLIENT: BATTELLE COLUMBUS LABS 20 DEC 85 :DATA
SAMPLE: ALUMINUM DUST I-9742 :JOB NUMBER

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE 20 DEC
SIZE-NORMALIZED VOLUME DISTRIBUTION
TOTAL =17152215

CHNL	SIZE	VOLUME	% >	CHNL	SIZE	VOLUME	% >	CHNL	SIZE	VOLUME	% >
1	.43	8189	100.00	43	1.84	101521	89.67	85	7.90	231949	42.19
2	.44	8860	99.95	44	1.91	115544	89.08	86	8.18	257276	40.34
3	.46	9578	99.90	45	1.97	121933	88.41	87	8.46	240147	39.34
4	.48	10347	99.84	46	2.04	124812	87.70	88	8.76	252798	37.71
5	.49	11167	99.78	47	2.12	126333	86.97	89	9.07	262143	36.43
6	.51	12042	99.72	48	2.19	132940	86.23	90	9.39	253948	34.91
7	.53	12973	99.65	49	2.27	136139	85.46	91	9.72	244977	33.42
8	.55	13966	99.57	50	2.35	140010	84.66	92	10.06	233806	32.00
9	.57	15021	99.49	51	2.43	141332	83.83	93	10.41	232022	30.62
10	.59	16143	99.40	52	2.52	143076	83.02	94	10.79	237750	29.27
11	.61	17333	99.31	53	2.60	162147	82.16	95	11.17	252052	27.88
12	.63	18595	99.21	54	2.70	166346	81.21	96	11.56	217381	26.42
13	.65	19932	99.10	55	2.79	166331	80.23	97	11.97	240643	25.15
14	.67	21347	98.98	56	2.89	173251	79.18	98	12.39	214896	23.74
15	.70	22842	98.86	57	2.99	180135	78.25	99	12.83	210001	22.49
16	.72	24422	98.73	58	3.10	180394	77.20	100	13.28	216855	21.27
17	.75	26089	98.58	59	3.21	181995	76.15	101	13.75	212248	20.00
18	.77	27847	98.43	60	3.32	208367	75.09	102	14.23	210336	18.77
19	.80	29697	98.27	61	3.44	197232	73.87	103	14.74	237453	17.54
20	.83	31644	98.10	62	3.56	205867	72.72	104	15.26	231000	16.15
21	.86	33688	97.91	63	3.68	174037	71.52	105	15.79	208552	14.81
22	.89	35835	97.72	64	3.81	205586	70.39	106	16.35	204406	13.59
23	.92	38089	97.51	65	3.95	202671	69.19	107	16.93	204542	12.40
24	.95	39649	97.29	66	4.09	210312	68.01	108	17.52	211485	11.21
25	.99	41644	97.05	67	4.23	216137	66.78	109	18.14	190093	9.97
26	1.02	44393	96.81	68	4.38	217849	65.52	110	18.78	174458	8.87
27	1.06	46022	96.55	69	4.54	227351	64.25	111	19.44	175101	7.85
28	1.10	49365	96.28	70	4.70	228991	62.92	112	20.13	172358	6.83
29	1.13	52445	95.99	71	4.86	227110	61.59	113	20.84	146806	5.82
30	1.17	53757	95.69	72	5.03	234218	60.27	114	21.57	131621	4.77
31	1.22	58185	95.37	73	5.21	237401	58.90	115	22.34	123410	4.20
32	1.26	60004	95.04	74	5.39	244957	57.52	116	23.12	116994	3.48
33	1.30	64339	94.69	75	5.58	242986	56.09	117	23.94	100777	2.80
34	1.35	68036	94.31	76	5.78	236533	54.67	118	24.78	94442	2.21
35	1.40	71661	93.91	77	5.98	236858	53.29	119	25.66	72248	1.66
36	1.45	78715	93.50	78	6.20	232698	51.91	120	26.56	59687	1.24
37	1.50	88249	93.04	79	6.41	234126	50.55	121	27.50	49415	.89
38	1.55	91558	92.52	80	6.64	241427	49.19	122	28.47	38927	.66
39	1.60	93171	91.99	81	6.87	224938	47.78	123	29.47	24134	.38
40	1.66	98710	91.44	82	7.12	235177	46.47	124	30.51	15933	.24
41	1.72	100631	90.87	83	7.37	251416	45.10	125	31.59	16998	.14
42	1.78	104134	90.26	84	7.63	246711	43.63	126	32.70	7449	.04

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE 16 JAN 85 :DATA
SAMPLE: POLYCRYSTALLINE IRON I-974 :JOB NUMBER

VOLUME (MASS) DISTRIBUTION FROM DISPLAY AREA: 4
==========
INDICES

VOLUME MODE = 70.19 MEDIAN = 66.67 MICRONS AND LARGER

GEOMETRIC VOLUME MEAN = 59.57 +/- 35.71 (59.95%) SKEWNESS = -.30

ARITHMETIC VOLUME MEAN = 65.02 +/- 23.02 (35.41%) SKEWNESS = -.22

PERCENTILE: 00.1% OF VOLUME IS AT 123.46 MICRONS AND LARGER
PERCENTILE: 01.0% OF VOLUME IS AT 117.28 MICRONS AND LARGER
PERCENTILE: 06.0% OF VOLUME IS AT 100.54 MICRONS AND LARGER
PERCENTILE: 22.0% OF VOLUME IS AT 81.87 MICRONS AND LARGER
PERCENTILE: 50.0% OF VOLUME IS AT 66.67 MICRONS AND LARGER
PERCENTILE: 78.0% OF VOLUME IS AT 50.27 MICRONS AND LARGER
PERCENTILE: 94.0% OF VOLUME IS AT 22.68 MICRONS AND LARGER
PERCENTILE: 99.0% OF VOLUME IS AT 11.05 MICRONS AND LARGER
PERCENTILE: 99.9% OF VOLUME IS AT 7.33 MICRONS AND LARGER

COUNT (FREQUENCY) DISTRIBUTION FROM DISPLAY AREA: 5
==========
INDICES

COUNTS MODE = 6.96 MEDIAN = 11.94 MICRONS AND LARGER

GEOMETRIC COUNTS MEAN = 14.01 +/- 12.37 (88.27%) SKEWNESS = .57

ARITHMETIC COUNTS MEAN = 17.92 +/- 16.00 (89.33%) SKEWNESS = .68

PERCENTILE: 00.1% OF COUNTS IS AT 103.15 MICRONS AND LARGER
PERCENTILE: 01.0% OF COUNTS IS AT 77.78 MICRONS AND LARGER
PERCENTILE: 06.0% OF COUNTS IS AT 54.29 MICRONS AND LARGER
PERCENTILE: 22.0% OF COUNTS IS AT 20.47 MICRONS AND LARGER
PERCENTILE: 50.0% OF COUNTS IS AT 11.94 MICRONS AND LARGER
PERCENTILE: 78.0% OF COUNTS IS AT 8.12 MICRONS AND LARGER
PERCENTILE: 94.0% OF COUNTS IS AT 7.14 MICRONS AND LARGER
PERCENTILE: 99.0% OF COUNTS IS AT 6.79 MICRONS AND LARGER
PERCENTILE: 99.9% OF COUNTS IS AT 6.61 MICRONS AND LARGER

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE 16 JAN 85 :DATA
SAMPLE: POLYCRYSTALLINE IRON I-974 :JOB NUMBER

PARTICLE SIZE VS. COUNTS
ENCLOSING
LOW AT 13 6.61 11 HIGH AT 124 114.31 1

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL COUNTS FROM CHANNEL 13 TO 124, AND SKIP: 2

% MAX	SIZE	0 10 20 30 40 50 60 70 80 90 100
4.3	6.61	
71.5	7.14	
62.1	7.72	
53.1	8.33	
48.4	9.00	
43.0	9.72	
41.4	10.50	
37.5	11.34	
35.9	12.25	
33.2	13.23	
32.0	14.29	
32.0	15.43	
28.9	16.67	
23.8	18.00	
23.0	19.44	
19.1	21.00	
16.0	22.68	
12.5	24.50	
11.3	26.46	
9.4	28.58	
7.4	30.87	
7.4	33.34	
5.5	36.01	
5.9	38.89	
7.0	42.00	
7.4	45.36	
7.4	49.00	
8.6	52.92	
8.6	57.16	
8.6	61.73	
8.6	66.67	
6.6	72.01	
5.1	77.78	
3.1	84.00	
1.2	90.73	
1.2	97.99	
.4	105.84	
.4	114.31	
% MAX	SIZE	0 10 20 30 40 50 60 70 80 90 100

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE 16 JAN 85 :DATA
SAMPLE: POLYCRYSTALLINE IRON I-974 :JOB NUMBER

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE 16 JAN
SIZE-NORMALIZED COUNTS DISTRIBUTION
TOTAL = 5829

CHNL	SIZE	COUNTS	% >	CHNL	SIZE	COUNTS	% >	CHNL	SIZE	COUNTS	% >
13	6.61	11	100.00	51	17.55	69	28.67	89	46.54	20	8.23
14	6.79	103	99.81	52	18.00	61	27.48	90	47.75	19	7.89
15	6.96	200	98.04	53	18.47	61	26.44	91	49.00	19	7.57
16	7.14	183	94.61	54	18.95	62	25.39	92	50.27	19	7.24
17	7.33	172	91.47	55	19.44	59	24.33	93	51.58	20	6.91
18	7.52	166	88.52	56	19.95	55	23.31	94	52.92	22	6.57
19	7.72	159	85.68	57	20.47	53	22.37	95	54.29	22	6.19
20	7.92	149	82.95	58	21.00	49	21.46	96	55.71	23	5.82
21	8.12	143	80.39	59	21.55	44	20.62	97	57.16	22	5.42
22	8.33	136	77.94	60	22.11	42	19.87	98	58.64	22	5.04
23	8.55	139	75.60	61	22.68	41	19.15	99	60.17	23	4.67
24	8.77	137	73.22	62	23.27	40	18.44	100	61.73	22	4.27
25	9.00	124	70.87	63	23.88	35	17.76	101	63.34	24	3.89
26	9.24	124	68.74	64	24.50	32	17.16	102	64.98	23	3.48
27	9.48	116	66.62	65	25.14	32	16.61	103	66.67	22	3.09
28	9.72	110	64.63	66	25.79	31	16.06	104	68.41	21	2.71
29	9.97	106	62.74	67	26.46	29	15.53	105	70.19	21	2.35
30	10.23	106	60.92	68	27.15	22	15.03	106	72.01	17	1.99
31	10.50	106	59.10	69	27.85	24	14.65	107	73.88	17	1.70
32	10.77	106	57.28	70	28.58	24	14.24	108	75.81	12	1.41
33	11.05	99	55.46	71	29.32	20	13.83	109	77.78	13	1.20
34	11.34	96	53.77	72	30.08	20	13.48	110	79.80	9	.98
35	11.64	103	52.12	73	30.87	19	13.14	111	81.87	9	.82
36	11.94	98	50.35	74	31.67	19	12.82	112	84.00	8	.67
37	12.25	92	48.67	75	32.49	20	12.49	113	86.19	6	.53
38	12.57	97	47.09	76	33.34	19	12.15	114	88.43	6	.43
39	12.89	90	45.43	77	34.20	18	11.82	115	90.73	3	.33
40	13.23	85	43.88	78	35.09	17	11.51	116	93.09	3	.27
41	13.57	85	42.43	79	36.01	14	11.22	117	95.51	2	.22
42	13.93	87	40.97	80	36.94	19	10.98	118	97.99	3	.19
43	14.29	82	39.48	81	37.90	19	10.65	119	100.54	2	.14
44	14.66	80	38.07	82	38.89	15	10.33	120	103.15	2	.10
45	15.04	77	36.70	83	39.90	18	10.07	121	105.84	1	.07
46	15.43	82	35.37	84	40.94	15	9.76	122	108.59	1	.05
47	15.83	77	33.97	85	42.00	18	9.50	123	111.41	1	.03
48	16.25	80	32.65	86	43.09	18	9.20	124	114.31	1	.02
49	16.67	74	31.27	87	44.21	19	8.89				
50	17.10	78	30.01	88	45.36	19	8.56				

DISPLAY AREA: 4

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE 16 JAN 85 :DATA
SAMPLE: POLYCRYSTALLINE IRON I-974 :JOB NUMBER

PARTICLE SIZE VS. VOLUME
ENCLOSING
LOW AT 12 6.45 158 HIGH AT 127 123.46 422374

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL VOLUME FROM CHANNEL 12 TO 127, AND SKIP: 2

% MAX	SIZE
.0	6.12
.0	6.61
.9	7.14
1.0	7.72
1.1	8.33
1.2	9.00
1.4	9.72
1.7	10.50
1.9	11.34
2.3	12.25
2.7	13.23
3.2	14.29
4.0	15.43
4.6	16.67
4.7	18.00
5.8	19.44
6.0	21.00
6.4	22.68
6.4	24.50
7.0	26.46
7.7	28.58
7.3	30.87
9.5	33.34
9.3	36.01
12.4	38.89
17.5	42.00
23.6	45.36
29.1	49.00
44.3	52.92
56.3	57.16
68.2	61.73
86.6	66.67
84.7	72.01
84.9	77.78
62.4	84.00
33.2	90.73
44.0	97.99
17.7	105.84
16.5	114.31
5.0	123.46

Scale: 0 10 20 30 40 50 60 70 80 90 100

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE 16 JAN 85 :DATA
SAMPLE: POLYCRYSTALLINE IRON I-974 :JOB NUMBER

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE 16 JAN
SIZE-NORMALIZED VOLUME DISTRIBUTION
TOTAL = 1.96491E 8

CHNL	SIZE	VOLUME	% >	CHNL	SIZE	VOLUME	% >	CHNL	SIZE	VOLUME	% >
12	6.45	158	100.00	51	17.55	419491	96.62	90	47.75	2306296	80.53
13	6.61	3746	100.00	52	18.00	398158	96.40	91	49.00	2444812	79.35
14	6.79	36351	100.00	53	18.47	434977	96.20	92	50.27	2690365	78.11
15	6.96	76006	99.98	54	18.95	472471	95.98	93	51.58	3040279	76.74
16	7.14	75441	99.94	55	19.44	485790	95.74	94	52.92	3719572	75.19
17	7.33	76306	99.90	56	19.95	498215	95.49	95	54.29	4017356	73.30
18	7.52	79245	99.86	57	20.47	512878	95.24	96	55.71	4440681	71.25
19	7.72	82167	99.82	58	21.00	506719	94.98	97	57.16	4722977	68.99
20	7.92	83101	99.78	59	21.55	500207	94.72	98	58.64	5140637	66.59
21	8.12	86391	99.74	60	22.11	516949	94.47	99	60.17	5637621	63.97
22	8.33	88994	99.70	61	22.68	537742	94.20	100	61.73	5719936	61.11
23	8.55	97957	99.65	62	23.27	570907	93.93	101	63.34	6975007	58.19
24	8.77	103946	99.60	63	23.88	549881	93.64	102	64.98	7049134	54.64
25	9.00	101971	99.55	64	24.50	533361	93.36	103	66.67	7264768	51.06
26	9.24	109980	99.50	65	25.14	566721	93.09	104	68.41	7720841	47.36
27	9.48	111277	99.44	66	25.79	598639	92.80	105	70.19	8000001	43.43
28	9.72	114060	99.38	67	26.46	588447	92.49	106	72.01	7102756	39.36
29	9.97	118910	99.32	68	27.15	502170	92.19	107	73.88	7748969	35.74
30	10.23	128220	99.26	69	27.85	588983	91.94	108	75.81	6064701	31.80
31	10.50	138713	99.20	70	28.58	643291	91.64	109	77.78	7122354	28.71
32	10.77	149081	99.13	71	29.32	563493	91.31	110	79.80	5181430	25.09
33	11.05	151218	99.05	72	30.08	613945	91.02	111	81.87	5703879	22.45
34	11.34	157890	98.98	73	30.87	611203	90.71	112	84.00	5230638	19.55
35	11.64	181652	98.90	74	31.67	678818	90.40	113	86.19	3891808	16.89
36	11.94	187854	98.80	75	32.49	753343	90.06	114	88.43	4067797	14.91
37	12.25	190280	98.71	76	33.34	799125	89.67	115	90.73	2782526	12.84
38	12.57	217191	98.61	77	34.20	776793	89.26	116	93.09	2736873	11.42
39	12.89	216923	98.50	78	35.09	788135	88.87	117	95.51	2220878	10.03
40	13.23	223392	98.39	79	36.01	778009	88.47	118	97.99	3690279	8.90
41	13.57	241278	98.28	80	36.94	1038013	88.07	119	100.54	2790004	7.02
42	13.93	266950	98.15	81	37.90	1174503	87.54	120	103.15	2152411	5.60
43	14.29	267155	98.02	82	38.89	1037891	86.95	121	105.84	1482549	4.50
44	14.66	282364	97.88	83	39.90	1270449	86.42	122	108.59	2008676	3.75
45	15.04	296963	97.74	84	40.94	1183824	85.77	123	111.41	1779255	2.73
46	15.43	339478	97.59	85	42.00	1467485	85.17	124	114.31	1386911	1.82
47	15.83	346415	97.41	86	43.09	1553586	84.42	125	117.28	796070	1.12
48	16.25	382557	97.24	87	44.21	1830508	83.63	126	120.33	974384	.71
49	16.67	388666	97.04	88	45.36	1977060	82.70	127	123.46	422374	.21
50	17.10	445283	96.84	89	46.54	2293516	81.69				

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PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 MAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 19 DEC 85 :DATA
SAMPLE: BRASS DUST I-9742 :JOB NUMBER

VOLUME (MASS) DISTRIBUTION FROM DISPLAY AREA: 4

INDICES

VOLUME MODE = 11.07 MEDIAN = 7.50 MICRONS AND LARGER

GEOMETRIC VOLUME MEAN = 6.09 +/- 8.56 (140.48%) SKEWNESS = -.58

ARITHMETIC VOLUME MEAN = 8.20 +/- 5.39 (65.72%) SKEWNESS = -.53

PERCENTILE: 00.1% OF VOLUME IS AT 25.22 MICRONS AND LARGER
PERCENTILE: 01.0% OF VOLUME IS AT 22.14 MICRONS AND LARGER
PERCENTILE: 06.0% OF VOLUME IS AT 17.83 MICRONS AND LARGER
PERCENTILE: 22.0% OF VOLUME IS AT 12.61 MICRONS AND LARGER
PERCENTILE: 50.0% OF VOLUME IS AT 7.50 MICRONS AND LARGER
PERCENTILE: 78.0% OF VOLUME IS AT 3.29 MICRONS AND LARGER
PERCENTILE: 94.0% OF VOLUME IS AT 1.16 MICRONS AND LARGER
PERCENTILE: 99.0% OF VOLUME IS AT .49 MICRONS AND LARGER
PERCENTILE: 99.9% OF VOLUME IS AT .28 MICRONS AND LARGER

COUNT (FREQUENCY) DISTRIBUTION FROM DISPLAY AREA: 5

INDICES

COUNTS MODE = .24 MEDIAN = .41 MICRONS AND LARGER

GEOMETRIC COUNTS MEAN = .47 +/- .36 (77.15%) SKEWNESS = .62

ARITHMETIC COUNTS MEAN = .58 +/- .62 (103.30%) SKEWNESS = .55

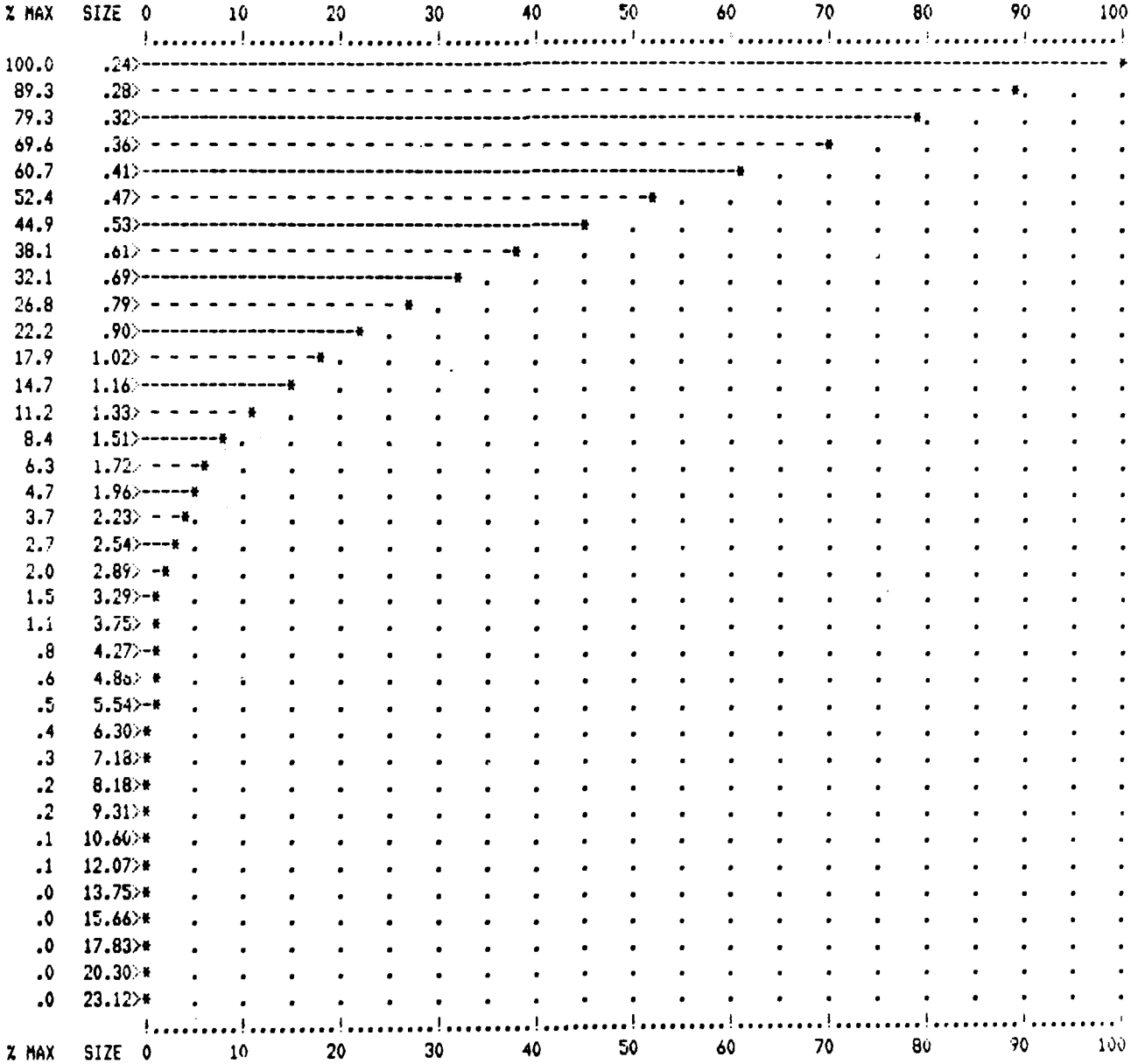
PERCENTILE: 00.1% OF COUNTS IS AT 7.50 MICRONS AND LARGER
PERCENTILE: 01.0% OF COUNTS IS AT 2.89 MICRONS AND LARGER
PERCENTILE: 06.0% OF COUNTS IS AT 1.33 MICRONS AND LARGER
PERCENTILE: 22.0% OF COUNTS IS AT .69 MICRONS AND LARGER
PERCENTILE: 50.0% OF COUNTS IS AT .41 MICRONS AND LARGER
PERCENTILE: 78.0% OF COUNTS IS AT .29 MICRONS AND LARGER
PERCENTILE: 94.0% OF COUNTS IS AT .26 MICRONS AND LARGER
PERCENTILE: 99.0% OF COUNTS IS AT .24 MICRONS AND LARGER
PERCENTILE: 99.9% OF COUNTS IS AT .24 MICRONS AND LARGER

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 19 DEC 85 :DATA
SAMPLE: BRASS DUST I-9742 :JOB NUMBER

PARTICLE SIZE VS. COUNTS
ENCLOSING
LOW AT 1 .24 65535 HIGH AT 106 23.12 1

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL COUNTS FROM CHANNEL 1 TO 106, AND SKIP: 2



PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 MAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 19 DEC 85 :DATA
SAMPLE: BRASS DUST I-9742 :JOB NUMBER

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE 19 DEC
SIZE-NORMALIZED COUNTS DISTRIBUTION
TOTAL = 1294908

CHNL	SIZE	COUNTS	% >	CHNL	SIZE	COUNTS	% >	CHNL	SIZE	COUNTS	% >
1	.24	65535	100.00	37	1.16	9661	8.25	73	5.54	330	.23
2	.26	63144	94.94	38	1.22	8830	7.51	74	5.78	296	.23
3	.27	60862	90.06	39	1.27	8098	6.82	75	6.04	283	.21
4	.28	58541	85.36	40	1.33	7336	6.20	76	6.30	265	.18
5	.29	56302	80.84	41	1.38	6724	5.63	77	6.58	240	.16
6	.30	54111	76.49	42	1.45	6034	5.11	78	6.87	216	.15
7	.32	51942	72.32	43	1.51	5490	4.65	79	7.16	199	.13
8	.33	49785	68.30	44	1.58	4951	4.22	80	7.50	179	.11
9	.35	47669	64.40	45	1.65	4540	3.84	81	7.83	162	.10
10	.36	45644	60.78	46	1.72	4112	3.49	82	8.16	143	.09
11	.38	43627	57.25	47	1.79	3765	3.17	83	8.54	132	.08
12	.39	41668	53.88	48	1.87	3385	2.88	84	8.92	115	.07
13	.41	39771	50.67	49	1.96	3078	2.62	85	9.31	106	.06
14	.43	37907	47.59	50	2.04	2871	2.38	86	9.72	92	.05
15	.45	36104	44.67	51	2.13	2611	2.16	87	10.15	83	.04
16	.47	34350	41.88	52	2.23	2400	1.96	88	10.60	71	.04
17	.49	32652	39.23	53	2.33	2132	1.77	89	11.07	64	.03
18	.51	30992	36.70	54	2.43	1950	1.61	90	11.56	57	.02
19	.53	29413	34.31	55	2.54	1758	1.46	91	12.07	46	.02
20	.56	27877	32.04	56	2.65	1593	1.32	92	12.61	42	.02
21	.58	26399	29.89	57	2.77	1439	1.20	93	13.17	36	.01
22	.61	24980	27.85	58	2.89	1297	1.09	94	13.75	30	.01
23	.63	23607	25.92	59	3.02	1175	.99	95	14.36	24	.01
24	.66	22290	24.10	60	3.15	1047	.90	96	14.99	21	.01
25	.69	21024	22.38	61	3.29	961	.82	97	15.66	16	.01
26	.72	19813	20.75	62	3.44	876	.74	98	16.35	13	.00
27	.75	18656	19.22	63	3.59	790	.67	99	17.07	10	.00
28	.79	17546	17.78	64	3.75	714	.61	100	17.83	9	.00
29	.82	16487	16.43	65	3.91	659	.56	101	18.62	6	.00
30	.86	15478	15.13	66	4.09	595	.51	102	19.44	4	.00
31	.90	14517	13.96	67	4.27	550	.46	103	20.30	4	.00
32	.94	13601	12.84	68	4.46	503	.42	104	21.20	3	.00
33	.98	12659	11.79	69	4.65	469	.38	105	22.14	1	.00
34	1.02	11764	10.81	70	4.86	418	.34	106	23.12	1	.00
35	1.07	11033	9.90	71	5.08	385	.31				
36	1.11	10365	9.05	72	5.30	357	.28				

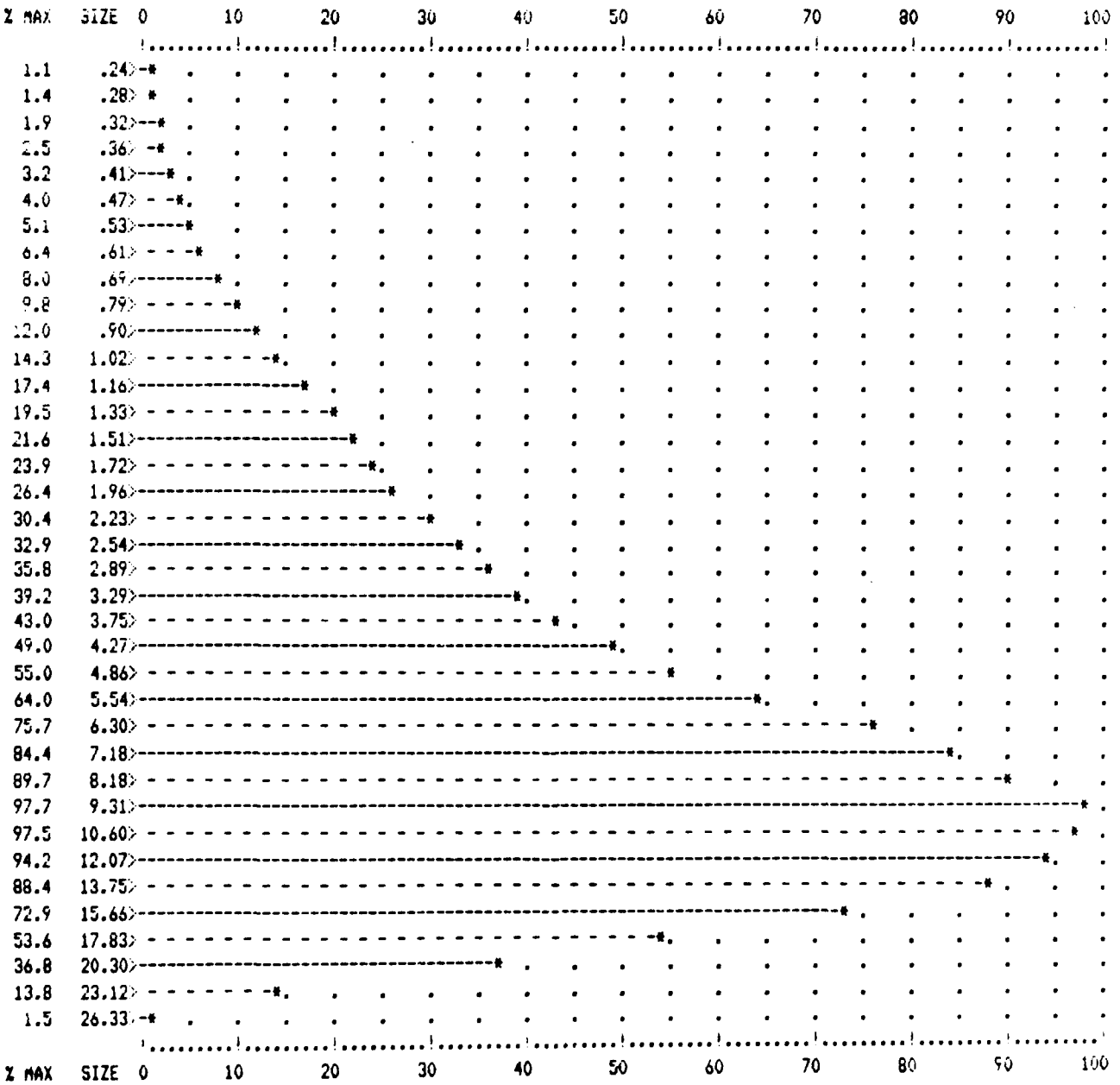
DISPLAY AREA: 4

PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 MAIN STREET - ELMHURST, IL. 60126
TELEPHONE: (312)832-5658

CLIENT: BATTELLE COLUMBUS LABS 19 DEC 85 :DATA
SAMPLE: BRASS DUST I-9742 :JOB NUMBER

PARTICLE SIZE VS. VOLUME
ENCLOSING
LOW AT 1 .24 720 HIGH AT 109 26.33 955

GRAPH OF DIAMETER SIZES VS. DIFFERENTIAL VOLUME FROM CHANNEL 1 TO 109, AND SKIP: 2



PARTICLE SIZE ANALYSIS BY ELZONE METHOD
PARTICLE DATA LABORATORIES, LTD.
115 HAHN STREET - ELMHURST, IL. 60126
TELEPHONE: (312/832-5658

CLIENT: BATTELLE COLUMBUS LABS 19 DEC 85 :DATA
SAMPLE: BRASS DUST I-9742 :JOB NUMBER

"TOTAL IN TABULATION= TOTAL COUNT OR VOLUME IN ANALYSIS

TABULATION

DATA ID 9742 DATE 19 DEC
SIZE-NORMALIZED VOLUME DISTRIBUTION
TOTAL = 2626877

CHNL	SIZE	VOLUME	% >	CHNL	SIZE	VOLUME	% >	CHNL	SIZE	VOLUME	% >
1	.24	720	100.00	38	1.22	11683	93.95	75	6.04	46649	60.48
2	.26	790	99.97	39	1.27	12410	93.49	76	6.30	49620	58.71
3	.27	867	99.94	40	1.33	12804	93.02	77	6.58	51258	56.82
4	.28	949	99.91	41	1.38	13366	92.53	78	6.87	52581	54.87
5	.29	1040	99.87	42	1.45	13659	92.03	79	7.18	55308	52.87
6	.30	1136	99.83	43	1.51	14150	91.51	80	7.50	56354	50.76
7	.32	1244	99.79	44	1.58	14532	90.97	81	7.83	58250	48.62
8	.33	1356	99.74	45	1.65	15177	90.41	82	8.18	58790	46.40
9	.35	1480	99.69	46	1.72	15853	89.84	83	8.54	62115	44.16
10	.36	1614	99.64	47	1.79	16322	89.24	84	8.92	60774	41.80
11	.38	1757	99.57	48	1.87	16705	88.62	85	9.31	64007	39.48
12	.39	1911	99.51	49	1.96	17301	87.98	86	9.72	63495	37.05
13	.41	2077	99.43	50	2.04	18385	87.32	87	10.15	64925	34.63
14	.43	2255	99.35	51	2.13	19037	86.62	88	10.60	63882	32.16
15	.45	2445	99.27	52	2.23	19725	85.90	89	11.07	65535	29.73
16	.47	2650	99.18	53	2.33	20130	85.14	90	11.56	64852	27.23
17	.49	2868	99.06	54	2.43	20992	84.37	91	12.07	61746	24.76
18	.51	3101	98.97	55	2.54	21569	83.58	92	12.61	63444	22.41
19	.53	3351	98.85	56	2.65	22244	82.75	93	13.17	60775	20.00
20	.56	3617	98.72	57	2.77	22881	81.91	94	13.75	57965	17.68
21	.58	3900	98.58	58	2.89	23478	81.04	95	14.36	54005	15.48
22	.61	4202	98.43	59	3.02	24222	80.14	96	14.99	50[illegible]	13.42
23	.63	4523	98.27	60	3.15	24588	79.22	97	15.66	477[illegible]	11.48
24	.66	4863	98.10	61	3.29	25697	78.26	98	16.35	42945	9.66
25	.69	5223	97.92	62	3.44	26690	77.31	99	17.07	39515	8.03
26	.72	5606	97.72	63	3.59	27378	76.29	100	17.83	35098	6.52
27	.75	6011	97.50	64	3.75	28171	75.25	101	18.62	31000	5.19
28	.79	6438	97.28	65	3.91	29618	74.18	102	19.44	27606	3.97
29	.82	6889	97.03	66	4.09	30492	73.05	103	20.30	24120	2.92
30	.86	7365	96.77	67	4.27	32101	71.89	104	21.20	19188	2.00
31	.90	7866	96.49	68	4.46	33369	70.67	105	22.14	13955	1.27
32	.94	8393	96.19	69	4.65	35496	69.40	106	23.12	9031	.74
33	.98	8950	95.87	70	4.86	36067	68.04	107	24.15	6260	.37
34	1.02	9365	95.53	71	5.08	37762	66.67	108	25.22	3134	.16
35	1.07	10054	95.17	72	5.30	39818	65.23	109	26.33	955	.04
36	1.11	10757	94.79	73	5.54	41962	63.72				
37	1.16	11417	94.3[illegible]	74	5.78	42951	62.12				

IMAGE ANALYSIS

ON

GRAPHITE FIBERS

DISTRIBUTIONS ON FREQUENCY AND MASS BASIS FOR:

LONGEST DIMENSION

BREADTH

ELONGATION RATIO L/D

EQUIVALENT CIRCULAR DIAMETER

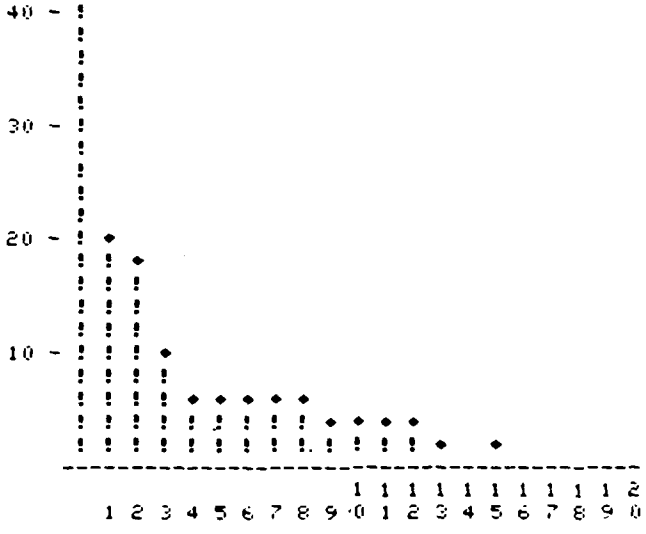
DISTRIBUTION LONG DIMEN

FEATURES	00442	FIELDS	00060
RANGE	+798.70	MIN	+10.65
TOTAL	+23853.92	MAX	+809.35
MEAN	+53.97	DEV	+88.37
MDIAN	+21.48	LOW	00452
		HIGH	00001

* Units are in micrometers

PDL Project: 1-9742

DIFFERENTIAL FREQUENCY DISTRIBUTION



Client: Battelle Columbus
Sample: Graphite Fibers
Date: December 26, 1985

CLASS INFORMATION

CLASS#	CLASS LIMITS		COUNT	# PERCENT
1	+10.00	+12.50	00092	+20.8
2	+12.50	+15.63	00079	+17.8
3	+15.63	+19.53	00040	+9.0
4	+19.53	+24.41	00025	+5.6
5	+24.41	+30.52	00027	+6.1
6	+30.52	+38.15	00023	+5.2
7	+38.15	+47.68	00023	+5.2
8	+47.68	+59.60	00025	+5.6
9	+59.60	+74.51	00022	+4.9
10	+74.51	+93.13	00018	+4.0
11	+93.13	+116.42	00017	+3.8
12	+116.42	+145.52	00019	+4.2
13	+145.52	+181.90	00012	+2.7
14	+181.90	+227.37	00004	+.9
15	+227.37	+284.22	00005	+1.1
16	+284.22	+355.27	00002	+.4
17	+355.27	+444.09	00004	+.9
18	+444.09	+555.11	00001	+.2
19	+555.11	+693.89	00002	+.4
20	+693.89	+867.36	00002	+.4

DISTRIBUTION LONG DIMEN

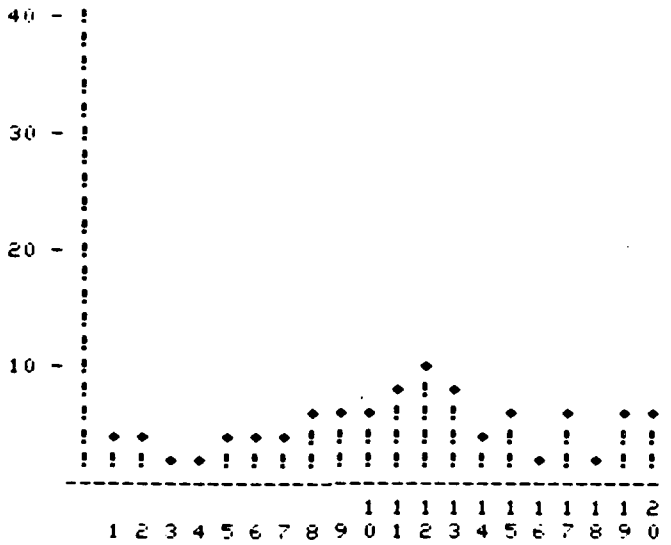
FEATURES	00442	FIELDS	00060
RANGE	+798.70	MIN	+10.65
TOTAL	+23853.92	MAX	+809.35
MEAN	+53.97	DEV	+88.37
MDIAN	+115.16	LOW	00452
		HIGH	00001

* Units are in micrometers

PDL Project: I-9742

Client: Battelle Columbus
Sample: Graphite Fibers
Date: December 26, 1985

DIFFERENTIAL WEIGHT DISTRIBUTION



CLASS INFORMATION

CLASS#	CLASS LIMITS		COUNT	WT PERCENT
1	+10.00 -	+12.50	00092	+4.2
2	+12.50 -	+15.63	00079	+4.6
3	+15.63 -	+19.53	00040	+2.9
4	+19.53 -	+24.41	00025	+2.2
5	+24.41 -	+30.52	00027	+3.0
6	+30.52 -	+38.15	00023	+3.2
7	+38.15 -	+47.68	00023	+4.0
8	+47.68 -	+59.60	00025	+5.5
9	+59.60 -	+74.51	00022	+6.1
10	+74.51 -	+93.13	00018	+6.2
11	+93.13 -	+116.42	00017	+7.3
12	+116.42 -	+145.52	00019	+10.3
13	+145.52 -	+181.90	00012	+8.1
14	+181.90 -	+227.37	00004	+3.3
15	+227.37 -	+284.22	00005	+5.3
16	+284.22 -	+355.27	00002	+2.6
17	+355.27 -	+444.09	00004	+6.6
18	+444.09 -	+555.11	00001	+2.0
19	+555.11 -	+693.89	00002	+5.1
20	+693.89 -	+867.36	00002	+6.4

DISTRIBUTION BREADTH

FEATURE:	00467	FIELD:	00060
RANGE	+26.03	MIN	+5.92
TOTAL	+4743.56	MAX	+31.95
MEAN	+10.16	DEV	+4.14

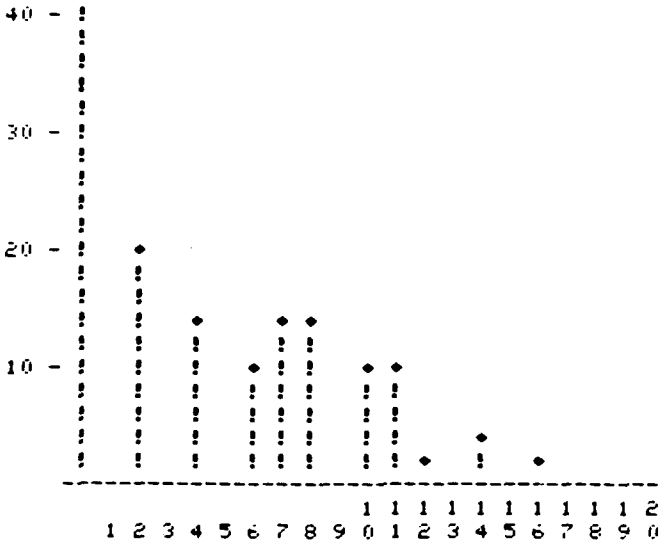
MDIAN +9.35 LOW 00422 HIGH 00006

* Units are in micrometers

PDL Project: I-9742

Client: Battelle Columbus
Sample: Graphite Fibers
Date: December 26, 1985

DIFFERENTIAL FREQUENCY DISTRIBUTION



CLASS INFORMATION

CLASS#	CLASS LIMITS		COUNT	# PERCENT
1	+5.00 -	+5.50	00000	000.0
2	+5.50 -	+6.05	00092	+19.7
3	+6.05 -	+6.65	00000	000.0
4	+6.65 -	+7.32	00061	+13.0
5	+7.32 -	+8.05	00000	000.0
6	+8.05 -	+8.86	00044	+9.4
7	+8.86 -	+9.74	00066	+14.1
8	+9.74 -	+10.72	00066	+14.1
9	+10.72 -	+11.79	00000	000.0
10	+11.79 -	+12.97	00045	+9.6
11	+12.97 -	+14.27	00043	+9.2
12	+14.27 -	+15.69	00014	+2.9
13	+15.69 -	+17.26	00004	+.8
14	+17.26 -	+18.99	00016	+3.4
15	+18.99 -	+20.89	00003	+.6
16	+20.89 -	+22.97	00006	+1.2
17	+22.97 -	+25.27	00002	+.4
18	+25.27 -	+27.80	00002	+.4
19	+27.80 -	+30.58	00001	+.2
20	+30.58 -	+33.64	00002	+.4

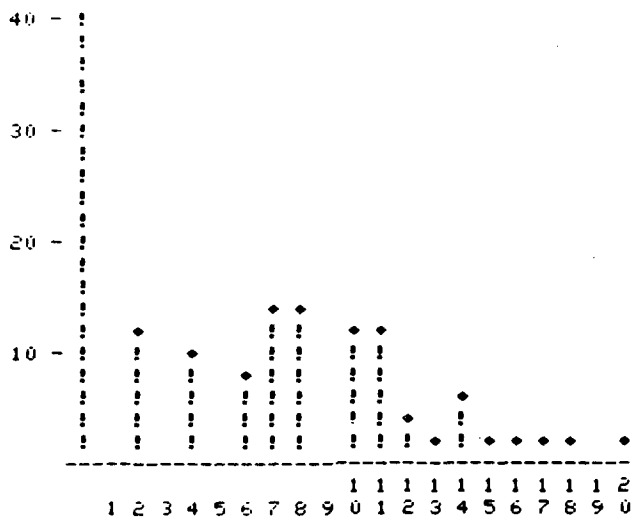
DISTRIBUTION BREADTH

FEATURES	00467	FIELDS	00060
RANGE	+26.03	MIN	+5.92
TOTAL	+4743.5*	MAX	+31.95
MEAN	+10.1*	DEV	+4.14
MDIAN	+10.3*	LOW	00422
		HIGH	00006

* Units are in micrometers

PDL Project: I-9742

DIFFERENTIAL WEIGHT DISTRIBUTION



Client: Battelle Columbus
Sample: Graphite Fibers
Date: December 24, 1985

CLASS INFORMATION

CLASS#	CLASS LIMITS		COUNT	WT PERCENT
1	+5.00 -	+5.50	00000	000.0
2	+5.50 -	+6.05	00092	+11.2
3	+6.05 -	+6.65	00000	000.0
4	+6.65 -	+7.32	00061	+9.0
5	+7.32 -	+8.05	00000	000.0
6	+8.05 -	+8.86	00044	+7.8
7	+8.86 -	+9.74	00066	+13.0
8	+9.74 -	+10.72	00066	+14.3
9	+10.72 -	+11.79	00000	000.0
10	+11.79 -	+12.97	00045	+11.8
11	+12.97 -	+14.27	00043	+12.4
12	+14.27 -	+15.69	00014	+4.4
13	+15.69 -	+17.26	00004	+1.3
14	+17.26 -	+18.99	00016	+6.1
15	+18.99 -	+20.89	00003	+1.2
16	+20.89 -	+22.97	00006	+2.7
17	+22.97 -	+25.27	00002	+1.0
18	+25.27 -	+27.80	00002	+1.1
19	+27.80 -	+30.58	00001	+.6
20	+30.58 -	+33.64	00002	+1.3

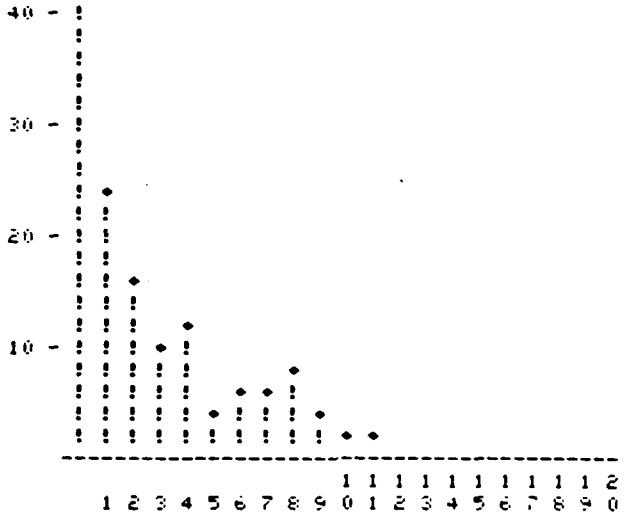
DISTRIBUTION EQ CIR DIA

FEATURE:	00622	FIELD:	00060
RANGE	+91.05	MIN	+5.11
TOTAL	+9879.87	MAX	+96.16
MEAN	+14.92	DEV	+13.01

* Units are in micrometers

MDIAN +9.80 LOW 00273 HIGH 00000

DIFFERENTIAL FREQUENCY DISTRIBUTION



PDL Project: 1-1742

Client: Battelle Columbus
Sample: Graphite Fibers
Date: December 24, 1985

CLASS INFORMATION

CLASS#	CLASS LIMITS		COUNT	# PERCENT
1	+5.00	+6.25	00145	+23.3
2	+6.25	+7.81	00100	+16.0
3	+7.81	+9.77	00065	+10.4
4	+9.77	+12.21	00079	+12.7
5	+12.21	+15.26	00031	+4.9
6	+15.26	+19.07	00043	+6.9
7	+19.07	+23.84	00041	+6.5
8	+23.84	+29.80	00049	+7.8
9	+29.80	+37.25	00031	+4.9
10	+37.25	+46.57	00018	+2.8
11	+46.57	+58.21	00010	+1.6
12	+58.21	+72.76	00005	+.8
13	+72.76	+90.95	00004	+.6
14	+90.95	+113.69	00001	+.1
15	+113.69	+142.11	00000	000.0
16	+142.11	+177.64	00000	000.0
17	+177.64	+222.04	00000	000.0
18	+222.04	+277.56	00000	000.0
19	+277.56	+346.94	00000	000.0
20	+346.94	+433.68	00000	000.0

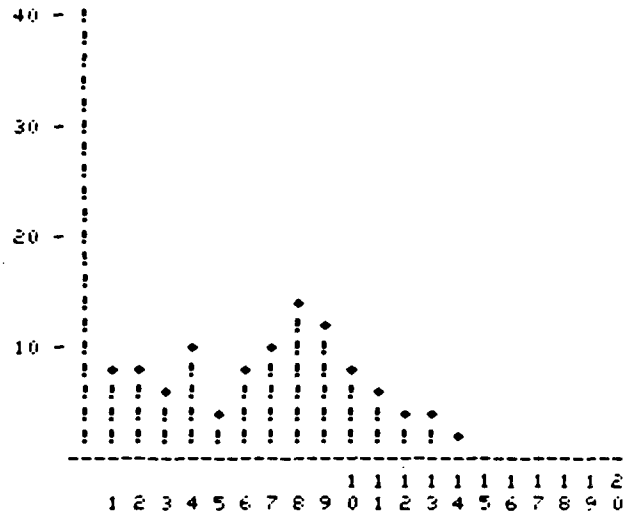
DISTRIBUTION EQ CIP DIA

FEATURE:	00622	FIELD:	00060
RANGE	+91.05	MIN	+5.11
TOTAL	+9279.87	MAX	+96.16
MEAN	+14.93	DEV	+13.01
MDIAN	+21.88	LOW 00273	HIGH 00000

* Units are in micrometers

PDL Project: I-9742

DIFFERENTIAL WEIGHT DISTRIBUTION



Client: Battelle Columbus
Sample: Graphite Fibers
Date: December 20, 1985

CLASS INFORMATION

CLASS#	CLASS LIMITS		COUNT	WT PERCENT
1	+5.00 -	+6.25	00145	+8.6
2	+6.25 -	+7.81	00100	+7.4
3	+7.81 -	+9.77	00065	+6.0
4	+9.77 -	+12.21	00079	+9.2
5	+12.21 -	+15.26	00031	+4.5
6	+15.26 -	+19.07	00043	+7.8
7	+19.07 -	+23.84	00041	+9.3
8	+23.84 -	+29.80	00049	+13.9
9	+29.80 -	+37.25	00031	+11.0
10	+37.25 -	+46.57	00018	+8.0
11	+46.57 -	+58.21	00010	+5.5
12	+58.21 -	+72.76	00005	+3.4
13	+72.76 -	+90.95	00004	+3.4
14	+90.95 -	+113.69	00001	+1.0
15	+113.69 -	+142.11	00000	000.0
16	+142.11 -	+177.64	00000	000.0
17	+177.64 -	+222.04	00000	000.0
18	+222.04 -	+277.56	00000	000.0
19	+277.56 -	+346.94	00000	000.0
20	+346.94 -	+433.68	00000	000.0

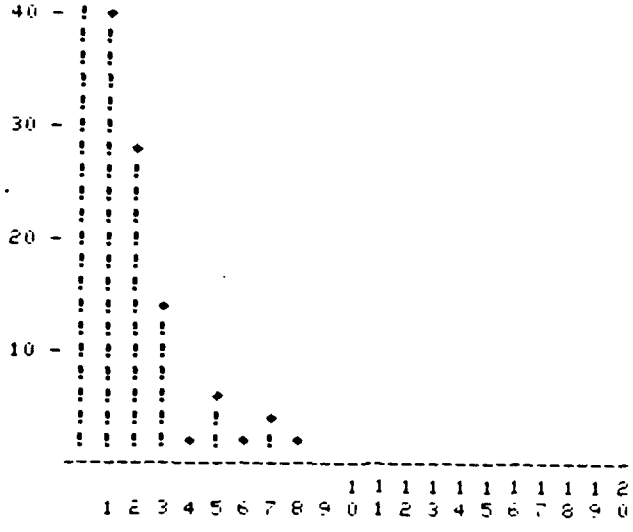
DISTRIBUTION ELONG RATIO

FEATURES	00689	FIELDS	00060
RANGE	+17.57	MIN	+1.12
TOTAL	+2148.50	MAX	+18.69
MEAN	+3.12	DEV	+2.60
MDIAN	+2.34	LOW 00000	HIGH 00006

PDL Project: 1-9742

Client: Battelle Columbus Labs
Sample: Graphite Fibers
Date: December 26, 1985

DIFFERENTIAL FREQUENCY DISTRIBUTION



CLASS INFORMATION

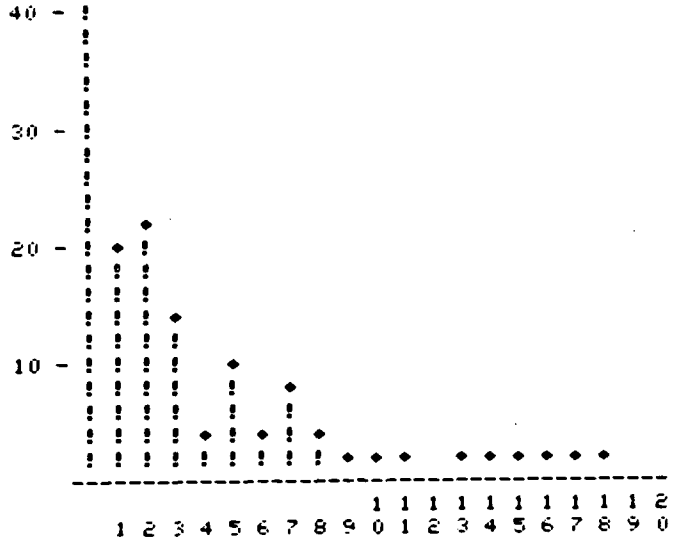
CLASS#	CLASS LIMITS		COUNT	# PERCENT
1	+1.00 -	+2.00	00281	+40.7
2	+2.00 -	+3.00	00187	+27.1
3	+3.00 -	+4.00	00090	+13.0
4	+4.00 -	+5.00	00020	+2.9
5	+5.00 -	+6.00	00040	+5.8
6	+6.00 -	+7.00	00013	+1.8
7	+7.00 -	+8.00	00022	+3.1
8	+8.00 -	+9.00	00011	+1.5
9	+9.00 -	+10.00	00005	+.7
10	+10.00 -	+11.00	00006	+.8
11	+11.00 -	+12.00	00002	+.2
12	+12.00 -	+13.00	00000	000.0
13	+13.00 -	+14.00	00002	+.2
14	+14.00 -	+15.00	00002	+.2
15	+15.00 -	+16.00	00002	+.2
16	+16.00 -	+17.00	00002	+.2
17	+17.00 -	+18.00	00002	+.2
18	+18.00 -	+19.00	00002	+.2
19	+19.00 -	+20.00	00000	000.0
20	+20.00 -	+21.00	00000	000.0

DISTRIBUTION ELONG RATIO

FEATURES	00689	FIELDS	00060
RANGE	+17.57	MIN	+1.12
TOTAL	+2148.50	MAX	+18.69
MEAN	+3.12	DEV	+2.60
MDIAN	+3.59	LOW 00000	HIGH 00006

DIFFERENTIAL WEIGHT DISTRIBUTION

PDL Project: I-9742



Client: Battelle Columbus
Sample: Graphite Fibers
Date: December 26, 1985

CLASS INFORMATION

CLASS#	CLASS LIMITS		COUNT	WT PERCENT
1	+1.00 -	+2.00	00281	+19.3
2	+2.00 -	+3.00	00187	+21.4
3	+3.00 -	+4.00	00090	+14.4
4	+4.00 -	+5.00	00020	+4.1
5	+5.00 -	+6.00	00040	+10.0
6	+6.00 -	+7.00	00013	+3.8
7	+7.00 -	+8.00	00022	+7.5
8	+8.00 -	+9.00	00011	+4.2
9	+9.00 -	+10.00	00005	+2.1
10	+10.00 -	+11.00	00006	+2.8
11	+11.00 -	+12.00	00002	+1.0
12	+12.00 -	+13.00	00000	000.0
13	+13.00 -	+14.00	00002	+1.2
14	+14.00 -	+15.00	00002	+1.3
15	+15.00 -	+16.00	00002	+1.4
16	+16.00 -	+17.00	00002	+1.5
17	+17.00 -	+18.00	00002	+1.6
18	+18.00 -	+19.00	00002	+1.6
19	+19.00 -	+20.00	00000	000.0
20	+20.00 -	+21.00	00000	000.0

IMAGE ANALYSIS

ON

NICKEL COATED GRAPHITE FIBERS

DISTRIBUTIONS ON FREQUENCY AND MASS BASIS FOR:

LONGEST DIMENSION

BREADTH

ELONGATION RATIO L/D

EQUIVALENT CIRCULAR DIAMETER

DISTRIBUTION LONG DIMEN

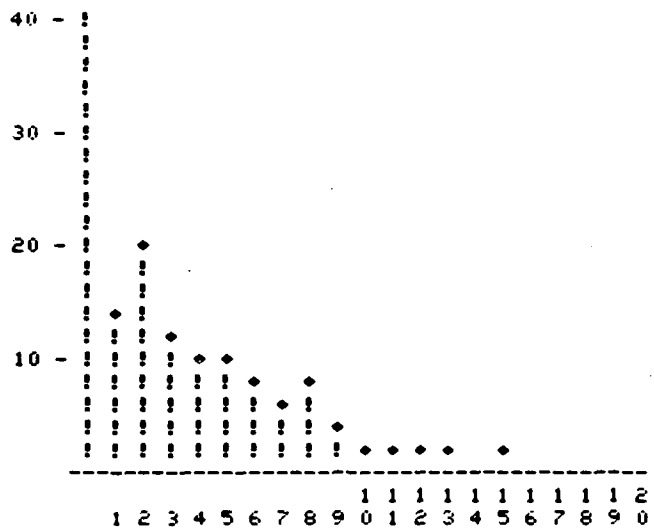
FEATURES	00450	FIELDS	00035
RANGE	+741.91	MIN	+10.65
TOTAL	+18909.06	MAX	+752.56
MEAN	+42.02	DEV	+65.11
MDIAN	+22.26	LOW	00325
		HIGH	00000

* Units are in micrometers

PDL Project: I-9742

Client: Battelle Columbus
Sample: Ni Graphite Fibers
Date: December 24, 1985

DIFFERENTIAL FREQUENCY DISTRIBUTION



CLASS INFORMATION

CLASS#	CLASS LIMITS		COUNT	# PERCENT
1	+10.00 -	+12.50	00060	+13.3
2	+12.50 -	+15.63	00090	+20.0
3	+15.63 -	+19.53	00051	+11.3
4	+19.53 -	+24.41	00043	+9.5
5	+24.41 -	+30.52	00044	+9.7
6	+30.52 -	+38.15	00032	+7.1
7	+38.15 -	+47.68	00030	+6.6
8	+47.68 -	+59.60	00035	+7.7
9	+59.60 -	+74.51	00014	+3.1
10	+74.51 -	+93.13	00009	+2.0
11	+93.13 -	+116.42	00013	+2.8
12	+116.42 -	+145.52	00011	+2.4
13	+145.52 -	+181.90	00006	+1.3
14	+181.90 -	+227.37	00000	000.0
15	+227.37 -	+284.22	00006	+1.3
16	+284.22 -	+355.27	00001	+.2
17	+355.27 -	+444.09	00002	+.4
18	+444.09 -	+555.11	00002	+.4
19	+555.11 -	+693.89	00000	000.0
20	+693.89 -	+867.36	00001	+.2

DISTRIBUTION LONG DIMEN

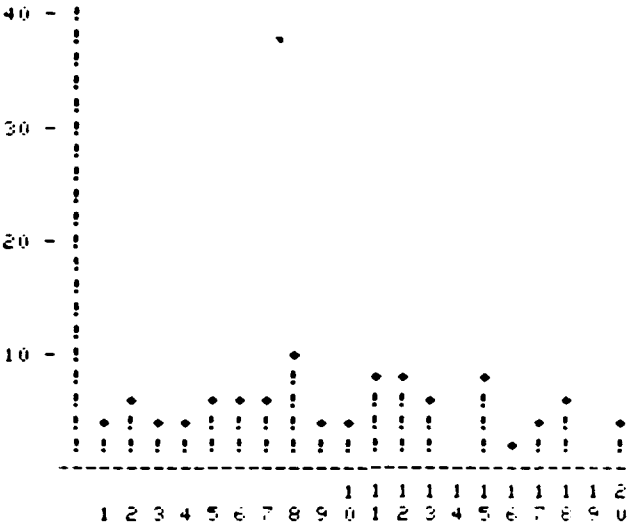
FEATURE:	00450	FIELD:	00035
RANGE	+741.91	MIN	+10.65
TOTAL	+18904.06	MAX	+752.56
MEAN	+42.02	DEV	+65.11

MDIAN +62.79 LOW 00325 HIGH 00000

* Units are in micrometers

PDL Project: I-9742

DIFFERENTIAL WEIGHT DISTRIBUTION



Client: Battelle Columbus
Sample: N. Grande Forks
Date: December 20, 1985

CLASS INFORMATION

CLASS#	CLASS LIMITS		COUNT	WT PERCENT
1	+10.00 -	+12.50	00060	+3.5
2	+12.50 -	+15.63	00090	+6.6
3	+15.63 -	+19.53	00051	+4.6
4	+19.53 -	+24.41	00043	+4.9
5	+24.41 -	+30.52	00044	+6.3
6	+30.52 -	+38.15	00036	+5.7
7	+38.15 -	+47.68	00030	+6.2
8	+47.68 -	+59.60	00025	+6.7
9	+59.60 -	+74.51	00014	+4.6
10	+74.51 -	+93.13	00009	+3.4
11	+93.13 -	+116.42	00001	+0.4
12	+116.42 -	+145.52	00011	+5.1
13	+145.52 -	+181.90	00006	+3.1
14	+181.90 -	+227.37	00000	00000.0
15	+227.37 -	+284.22	00002	+1.2
16	+284.22 -	+355.27	00001	+1.0
17	+355.27 -	+444.09	00001	+1.1
18	+444.09 -	+555.11	00000	+0.0
19	+555.11 -	+693.89	00000	+0.0
20	+693.89 -	+867.36	00001	+1

DISTRIBUTION BREADTH

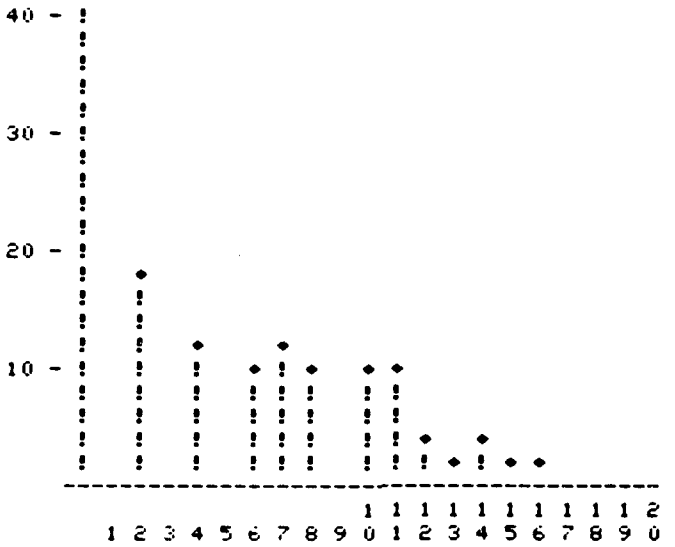
FEATURES	00481	FIELDS	00035
RANGE	+27.82	MIN	+5.92
TOTAL	+5234.53	MAX	+33.13
MEAN	+10.88	DEV	+4.78

MDIAN +9.60 LOW 00288 HIGH 00006

* Units are in micrometers

PDL Project: I-9742

DIFFERENTIAL FREQUENCY DISTRIBUTION



Client: Battelle Columbus
Sample: NC Graphite Fibers
Date: December 26, 1985

CLASS INFORMATION

CLASS#	CLASS LIMITS		COUNT	# PERCENT
1	+5.00 -	+5.50	00000	000.0
2	+5.50 -	+6.05	00086	+17.8
3	+6.05 -	+6.65	00000	000.0
4	+6.65 -	+7.32	00060	+12.4
5	+7.32 -	+8.05	00000	000.0
6	+8.05 -	+8.86	00050	+10.3
7	+8.86 -	+9.74	00053	+11.0
8	+9.74 -	+10.72	00047	+9.7
9	+10.72 -	+11.79	00000	000.0
10	+11.79 -	+12.97	00048	+9.9
11	+12.97 -	+14.27	00052	+10.8
12	+14.27 -	+15.69	00022	+4.5
13	+15.69 -	+17.26	00013	+2.7
14	+17.26 -	+18.99	00022	+4.5
15	+18.99 -	+20.89	00005	+1.0
16	+20.89 -	+22.97	00013	+2.7
17	+22.97 -	+25.27	00002	+.4
18	+25.27 -	+27.80	00004	+.8
19	+27.80 -	+30.58	00000	000.0
20	+30.58 -	+33.64	00004	+.8

DISTRIBUTION BREADTH

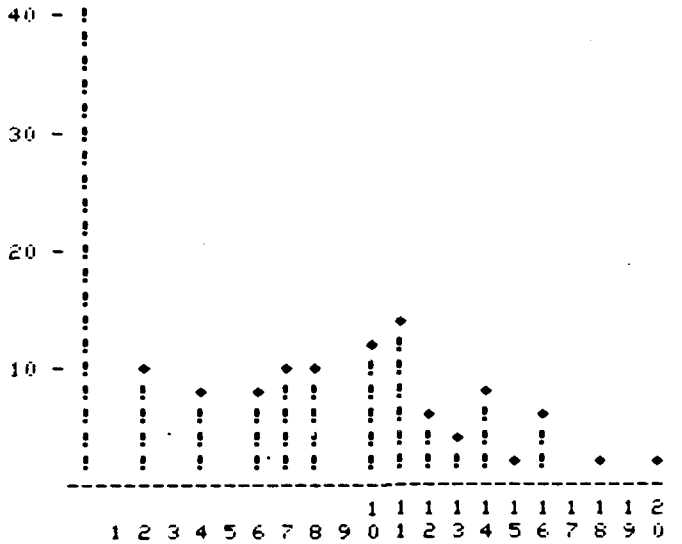
FEATURES	00481	FIELDS	00035
RANGE	+27.22	MIN	+5.92
TOTAL	+5234.53	MAX	+33.13
MEAN	+10.88	DEV	+4.78
MDIAN	+12.39	LOW 00288	HIGH 00006

* Units are in micrometers

PDL Project: I-9742

Client: Battelle Columbus
Sample: N. Graphite Fibers
Date: December 26, 1985

DIFFERENTIAL WEIGHT DISTRIBUTION



CLASS INFORMATION

CLASS#	CLASS LIMITS		COUNT	WT PERCENT
1	+5.00 -	+5.50	00000	000.0
2	+5.50 -	+6.05	00086	+9.5
3	+6.05 -	+6.65	00000	000.0
4	+6.65 -	+7.32	00060	+8.0
5	+7.32 -	+8.05	00000	000.0
6	+8.05 -	+8.86	00050	+8.0
7	+8.86 -	+9.74	00053	+9.4
8	+9.74 -	+10.72	00047	+9.2
9	+10.72 -	+11.79	00000	000.0
10	+11.79 -	+12.97	00048	+11.3
11	+12.97 -	+14.27	00052	+13.5
12	+14.27 -	+15.69	00022	+6.3
13	+15.69 -	+17.26	00013	+4.0
14	+17.26 -	+18.99	00022	+7.6
15	+18.99 -	+20.89	00005	+1.9
16	+20.89 -	+22.97	00013	+5.4
17	+22.97 -	+25.27	00002	+.9
18	+25.27 -	+27.80	00004	+2.0
19	+27.80 -	+30.58	00000	000.0
20	+30.58 -	+33.64	00004	+2.4

DISTRIBUTION EQ CIR DIA

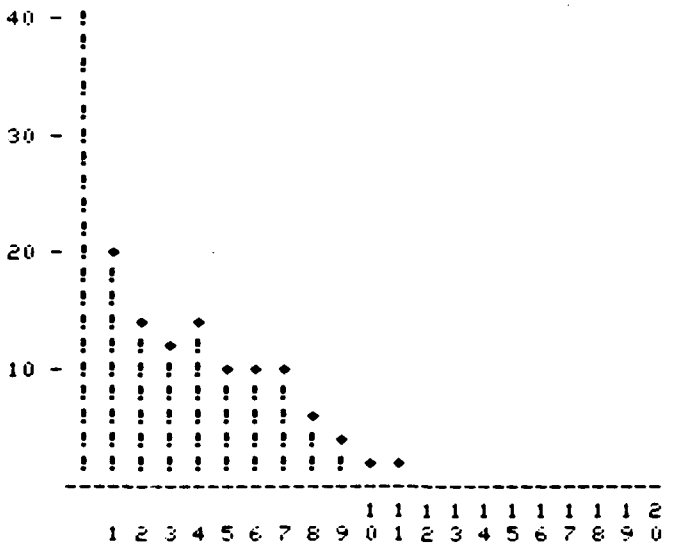
FEATURE:	00609	FIELD:	00035
RANGE	+98.24	MIN	+5.11
TOTAL	+8716.28	MAX	+103.35
MEAN	+14.31	DEV	+11.05
MDIAN	+10.65	LOW 00166	HIGH 00000

* Units are in micrometers

PDL Project: I-9742

Client: Battelle Columbus
Sample: Ni Graphite Fibers
Date: December 26, 1985

DIFFERENTIAL FREQUENCY DISTRIBUTION



CLASS INFORMATION

CLASS#	CLASS LIMITS		COUNT	# PERCENT
1	+5.00 -	+6.25	00117	+19.2
2	+6.25 -	+7.81	00082	+13.4
3	+7.81 -	+9.77	00075	+12.3
4	+9.77 -	+12.21	00084	+13.7
5	+12.21 -	+15.26	00056	+9.1
6	+15.26 -	+19.07	00055	+9.0
7	+19.07 -	+23.84	00055	+9.0
8	+23.84 -	+29.80	00035	+5.7
9	+29.80 -	+37.25	00022	+3.6
10	+37.25 -	+46.57	00014	+2.2
11	+46.57 -	+58.21	00008	+1.3
12	+58.21 -	+72.76	00005	+.8
13	+72.76 -	+90.95	00000	000.0
14	+90.95 -	+113.69	00001	+.1
15	+113.69 -	+142.11	00000	000.0
16	+142.11 -	+177.64	00000	000.0
17	+177.64 -	+222.04	00000	000.0
18	+222.04 -	+277.56	00000	000.0
19	+277.56 -	+346.94	00000	000.0
20	+346.94 -	+433.68	00000	000.0

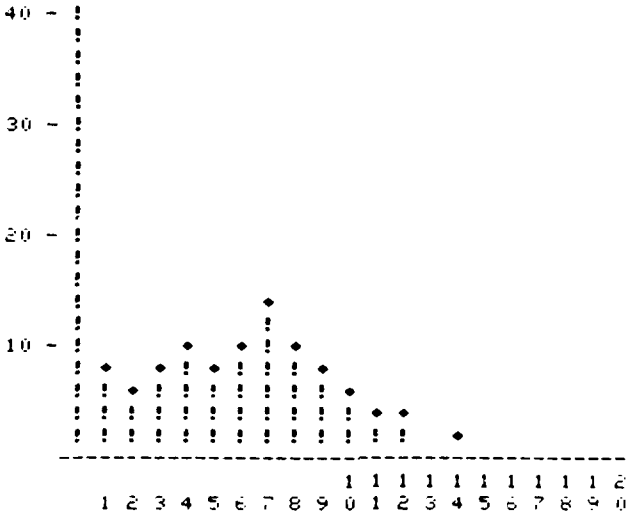
DISTRIBUTION EQ CIR DIA * Units are in micrometers

FEATURES	00609	FIELDS	00035
RANGE	+98.24	MIN	+5.11
TOTAL	+8716.38	MAX	+103.35
MEAN	+14.31	DEV	+11.05
MDIAN	+18.38	LOW 00166	HIGH 00000

PDL Project: I-9742

Client: Battelle Columbus
Sample: N. Graphite Fibers
Date: December 26, 1985

DIFFERENTIAL WEIGHT DISTRIBUTION



CLASS INFORMATION

CLASS#	CLASS LIMITS		COUNT	WT PERCENT
1	+5.00 -	+6.25	00117	+7.4
2	+6.25 -	+7.81	00082	+6.5
3	+7.81 -	+9.77	00075	+7.4
4	+9.77 -	+12.21	00084	+10.4
5	+12.21 -	+15.26	00056	+8.7
6	+15.26 -	+19.07	00055	+10.7
7	+19.07 -	+23.84	00055	+13.3
8	+23.84 -	+29.80	00035	+10.6
9	+29.80 -	+37.25	00022	+8.3
10	+37.25 -	+46.57	00014	+6.6
11	+46.57 -	+58.21	00008	+4.7
12	+58.21 -	+72.76	00005	+3.7
13	+72.76 -	+90.95	00000	000.0
14	+90.95 -	+113.69	00001	+1.1
15	+113.69 -	+142.11	00000	000.0
16	+142.11 -	+177.64	00000	000.0
17	+177.64 -	+222.04	00000	000.0
18	+222.04 -	+277.56	00000	000.0
19	+277.56 -	+346.94	00000	000.0
20	+346.94 -	+433.68	00000	000.0

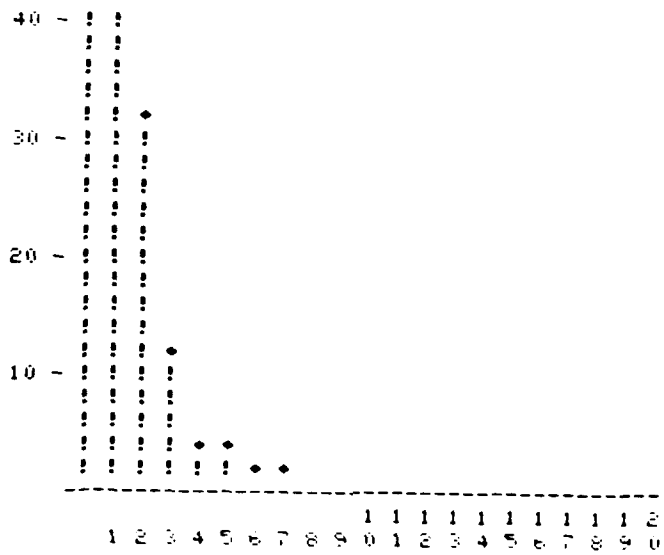
DISTRIBUTION ELONG RATIO

FEATURE:	01084	FIELD:	00052
RANGE	+19.17	MIN	+1.12
TOTAL	+2877.31	MAX	+20.30
MEAN	+2.65	DEV	+1.92
MDIAN	+2.22	LOW 00000	HIGH 00003

PDL Project: 1-9742

Client: Battelle Columbus
Sample: N. Graphite Fibers
Date: December 26, 1985

DIFFERENTIAL FREQUENCY DISTRIBUTION



CLASS INFORMATION

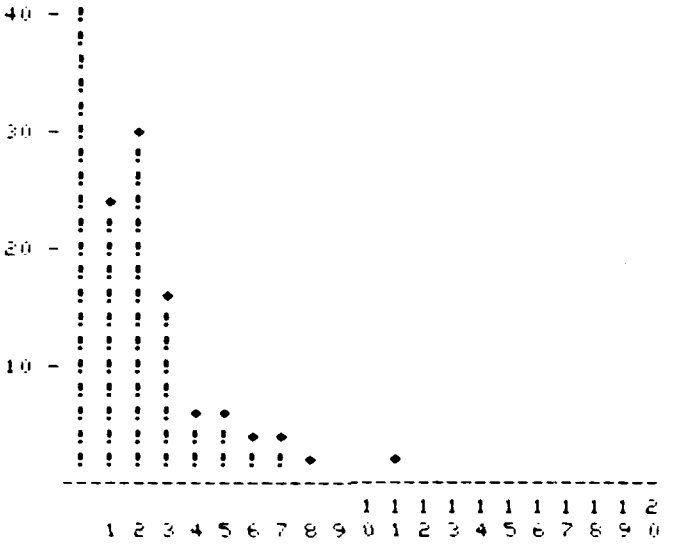
CLASS#	CLASS LIMITS		COUNT	% PERCENT
1	+1.00 -	+2.00	00465	+42.8
2	+2.00 -	+3.00	00356	+32.8
3	+3.00 -	+4.00	00132	+12.1
4	+4.00 -	+5.00	00041	+3.7
5	+5.00 -	+6.00	00034	+3.1
6	+6.00 -	+7.00	00020	+1.8
7	+7.00 -	+8.00	00013	+1.1
8	+8.00 -	+9.00	00008	+.7
9	+9.00 -	+10.00	00001	000.0
10	+10.00 -	+11.00	00002	+.1
11	+11.00 -	+12.00	00005	+.4
12	+12.00 -	+13.00	00001	000.0
13	+13.00 -	+14.00	00000	000.0
14	+14.00 -	+15.00	00001	000.0
15	+15.00 -	+16.00	00001	000.0
16	+16.00 -	+17.00	00001	000.0
17	+17.00 -	+18.00	00000	000.0
18	+18.00 -	+19.00	00001	000.0
19	+19.00 -	+20.00	00001	000.0
20	+20.00 -	+21.00	00001	000.0

DISTRIBUTION ELONG RATIO

FEATURES	01084	FIELDS	00052
RANGE	+19.17	MIN	+1.12
TOTAL	+2877.31	MAX	+20.30
MEAN	+2.65	DEV	+1.92
MDIAN	+2.83	LOW 00000	HIGH 00003

PDL Project: I-9742

DIFFERENTIAL WEIGHT DISTRIBUTION



Client: Sandia National Laboratories
Sample: Natural Fines
Date: [illegible]

CLASS INFORMATION

CLASS #	CLASS LIMITS	COUNT	WT PERCENT
1	+1.00 - +2.00	00465	+23.8
2	+2.00 - +3.00	00596	+30.4
3	+3.00 - +4.00	00132	+15.8
4	+4.00 - +5.00	00041	+6.3
5	+5.00 - +6.00	00034	+6.3
6	+6.00 - +7.00	00020	+4.4
7	+7.00 - +8.00	00013	+3.3
8	+8.00 - +9.00	00008	+2.3
9	+9.00 - +10.00	00001	+.3
10	+10.00 - +11.00	00002	+.7
11	+11.00 - +12.00	00005	+1.6
12	+12.00 - +13.00	00001	+.4
13	+13.00 - +14.00	00000	0000.0
14	+14.00 - +15.00	00001	+.4
15	+15.00 - +16.00	00001	+.5
16	+16.00 - +17.00	00001	+.5
17	+17.00 - +18.00	00000	0000.0
18	+18.00 - +19.00	00001	+.4
19	+19.00 - +20.00	00001	+.4
20	+20.00 - +21.00	00001	+.4

PARTICLE SIZE ANALYSIS AND SILICA DETERMINATIONS

The following is a description of the methods and procedures used to determine the particle size distribution of the test materials by either the Elzone analysis method or by image analysis.

Also included is the Standard Operating Procedure for silica determination.

PARTICLE DATA LABORATORIES, LTD.

115 Hahn Street • Elmhurst, Illinois 60126 • (312) 832-5658

17 January 86

Battelle Memorial Institute
Columbus Laboratory
505 King Avenue
Columbus Ohio
43201

Attn: Dr. Michael Placke

Subject: Elzone Analysis of Particulate Matter

PDL Project: I-9742

Dear Dr. Placke,

We have completed the Elzone analysis of the two difficult samples that you have recently submitted. As we have discussed, the Polycrystalline Iron sample had been dispersed in pure honey and then analyzed in a 4% pyrophosphate solution. A dilute suspension was used so that a reagglomeration would not occur due to magnetic forces.

We are happy with the results of the analysis because the data indicates that a bimodal situation does exist in the sample. Microscope examination reveals that is in fact the case. The sample is composed mainly of long interwoven iron fibers and the subpopulation consists of non fiber like residue extending down to submicron sizes.

We analyzed the Crocidolite down to 0.78 microns directly and then called a standard output. Following the reporting of that data, we instructed the computer to extrapolate the remainder of the distribution based upon the available information and assuming that the data was based upon a log-normal populaton. A second standard output was called and we have included that for your consideration.

If you have any questions concerning the data or the techniques used to generate the information, please do not hesitate to contact us at Particle Data Labs.

Sincelely,

Richard Karuhn
Director

PARTICLE DATA LABORATORIES, LTD.

115 Hahn Street • Elmhurst, Illinois 60126 • (312) 832-5658

June 2, 1987

Battelle Memorial Institute
Columbus Laboratories
505 King Avenue
Columbus, Ohio
43201

ATTN: Dr. Michael Placke

Subject: Sample Preparation Procedures Used for Samples Furnished Under PDL Project I-9742.

Gentlemen:

On January 17, 1986, Particle Data Laboratories reported the results of particle size data obtained from several samples using the computerized Elzone analyzer and our Bausch and Lomb Omnicon 5000 Image Analysis System. We have now been requested to furnish the sample preparation procedures for these samples on both instruments.

ELZONE ANALYZER

All samples were suspended in a 4% by weight sodium pyrophosphate solution prefiltered through a 0.45 micron Gelman filter capsule. Each sample was wetted in an appropriate surfactant prior to ultrasonic treatment in a standard laboratory ultrasonic unit. Following the deagglomeration step, a portion of the suspension was removed for microscopic evaluation. The purpose of this step was to determine the size of the largest particle present in the sample so a detection aperture of appropriate size could be placed on the analytical unit.

The analysis is always started from the large end of the sample and the the fine end of the distribution is determined by changing the size of the detection orifice on the Elzone unit. The individual parts of the distribution are then computer blended into one continuous curve and then the data is automatically printed.

The following table lists the surfactants and the size of the detection apertures used for each individual sample.

SURFACTANTS AND DETECTION APERTURES

SAMPLE I.D.	SURFACTANT	DETECTION ORIFICE DIAMETER (microns)
ALUMINUM DUST	TWEEN 20	120, 76, 24
MICRO 260 GRAPHITE	LOMAR PW	76, 24
DIXON KS-2 NATURAL GRAPHITE	LOMAR PW	76, 24
BRASS DUST	TWEEN 20	76, 24
CARBON BLACK	LOMAR PW	240, 76, 24

IMAGE ANALYSIS

The sample prep required for image analysis is considerably easier than a standard Elzone run because the samples can be dispersed in glycerine or an oil of higher refractive index than the sample. Once the dispersion on the microscope slide is complete, then the technician must dilute the concentration so that particles do not touch each other. If this were to happen then the analyzer would size the particles as one and a biased analysis would result.

Following the dispersion and dilution step, the image analyzer is programed to automatically step through a "search" pattern, focus and process the image in the field of view. Following the collection of image data, report generation is automatically conducted by the system from internal routines. We simply instruct the instrument which shape parameters we want printed.

CONCLUSION

The above information was compiled from data retained in our project file I-9742. If there is any additional information that you need concerning this project, please do not hesitate to contact us at Particle Data Laboratories.

Sincerely,

Richard Karuhn

Richard Karuhn
Director

PARTICLE DATA LABORATORIES, LTD.

115 Hahn Street • Elmhurst, Illinois 60126 • (312) 832-5658

BASIC ELECTROZONE TECHNOLOGY

AND

EXPLANATION OF REPORT

The electric sensing zone analytical technique has developed rapidly over the past twenty years. In this technique, particles suspened in a conductive fluid, flow serially through an orifice under a differential pressure. Electrodes are immersed on each side of the orifice as shown in Figure 1. As each particle passes through the aperture, it replaces its own volume of electrolyte within the aperture, momentarily changing the resistance value between the electrodes. This change produces a voltage pulse of short duration having a magnitude proportional to particle volume. The resulting series of pulses is electronically amplified, scaled and counted. Raw data processing is performed by a PDP-1103 minicomputer in such a manner that a population histogram of 128 or 256 channels of information can be acquired. Acquired data is conditioned by applying calibration, extrapolation, volume (weight) or area conversions. Normalization of size and quantity axes to the types of scales required by the researcher is also possible.

The conductive particle suspension medium is an important consideration in Electrozone technology. Typically, aquesous isotonic saline (0.9% by weight) or a 4% by weight sodium pyrophosphate is used as a dispersing and particle suspension medium. For certain analyses which cannot be run in an aqueous media, 4% weight/volume lithium chloride in isopropyl alcohol is effective.

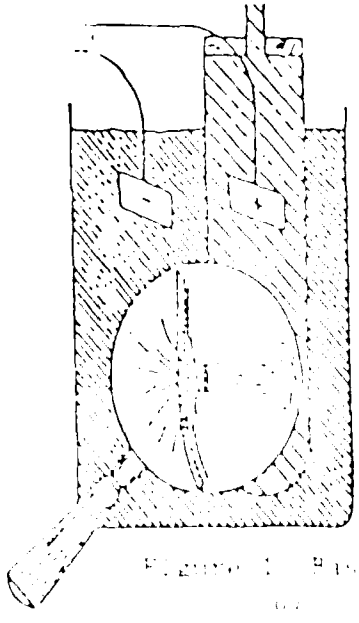


Figure IIA is a cross section of the orifice shown in Figure 1. In this configuration, no particle is shown in the orifice. Since a constant current is established in the conductive liquid and through the sapphire orifice, a constant voltage potential is represented as the product of the current (I) and Resistance (R).

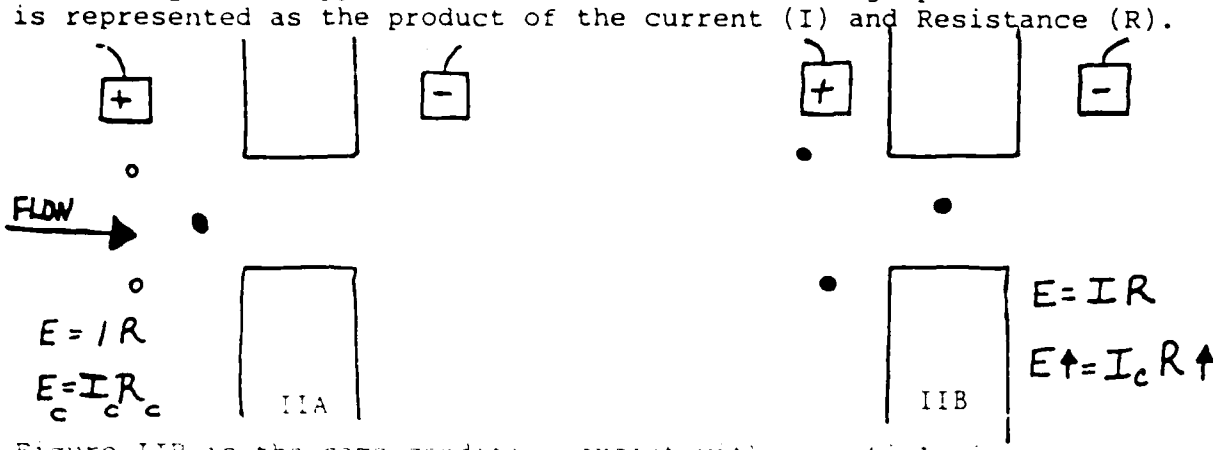
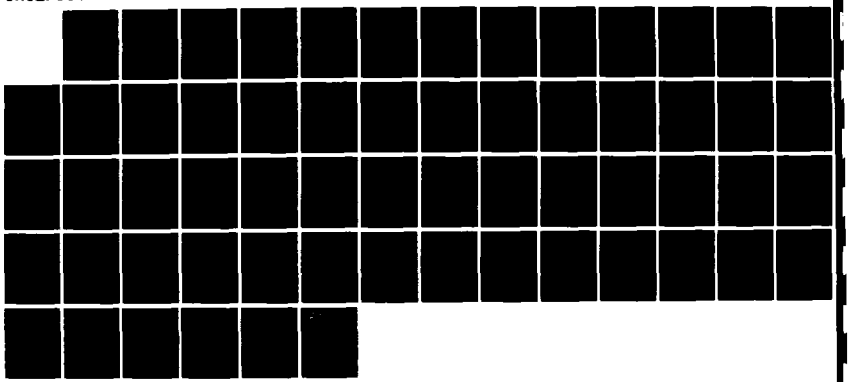


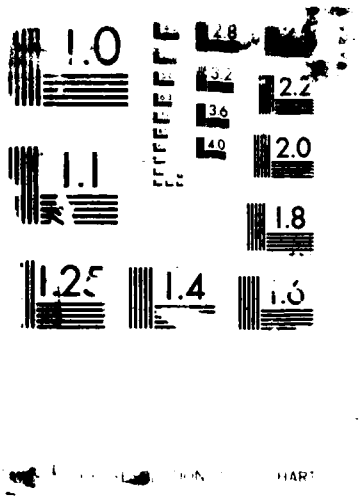
Figure IIB is the same condition except with a particle in the orifice or sensing zone. Since almost all particles act as insulators, the electrical resistance increases in the orifice. Under the conditions of a constant current and increased resistance the product of these two must rise according to Ohm's Law. Since the particles traverse the orifice in about 20 micro seconds, a voltage pulse is produced. The magnitude of this voltage pulse is proportional to the envelope volume of the particle. That is, a small particle yields a small voltage pulse while a large particle yeilds a large pulse. The particle may be irregular in shape (spheres are seldom encountered), but since the volume of that particle has been measured, the diameter of a sphere of equal volume can be assigned to that particle. This method of expressing data is the "Diameter of a Sphere of Equal Volume" is used throughout all Particle Technology."

Now that we have a way to measure discrete events very rapidly and accurately, it is necessary to present a representative population of the powder. The term "representative" means that the sample the powder is a miniature of the powder and that it represents the powder as that property is measured by the instrument. When all factors have been met, the suspension is analyzed by counting and sizing not fewer than fifty thousand particles in a sample. Since the accuracy and precision of the measurement is also tied to the sample size, we elect to count such a large number of particles. When a preset number of particles has been analyzed, the computer stops the analysis. At this point permanent calibration information is called from the keyboard and the frequency population statistics are generated. The information is then converted to a volume (mass) basis and these statistics are reported.

AD-A189 829 IN VITRO TOXICITY EVALUATION OF TEN PARTICULATE MATERIALS IN TRACHEAL ORGAN CULTURE(U) BATTELLE COLUMBUS DIV OH M E PLACKE ET AL. DEC 87 2/2

UNCLASSIFIED CRDEC-CR-88018 DAAK11-82-D-0008 F/G 6/11 NL




1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.8
3.2
3.6
4.0

PARTICLE DATA LABORATORIES, LTD.

There are two classic methods of fine particle size analysis:

1. Frequency Distribution (Microscope Counting)

2. Mass Distribution (Sieves of Andreason sedimentation)

In the first method, the number of particles of a specific size are tabulated by the microscopist. He scans a microscope slide while randomly searching for a particle in the prepared slide. When one is located, it is sized using an eyepiece micrometer and counted as a frequency of occurrence. Soon a frequency distribution is established for the sample of interest. The microscopist can now calculate the relative percent of particles within a given size interval or he can sum the data and report the percentage greater than an indicated size. Table I is a brief example of this precedure. Following the statistical treatment, he can plot the data to locate the geometric median diameter and then derive other statistical parameters.

Table I

Example of Frequency Distribution Data

(μm) Particle Size Interval	d Mid Size	N Frequency of Occurance	N≥ Cumulative Frequency	Cumulative Frequency ≥Indicated%
1.0 - 1.4	1.2	10	100	100
1.4 - 2.0	1.7	15	90	90
2.0 - 2.8	2.4	50	75	75
2.8 - 4.0	3.2	15	25	25
4.0 - 5.6	4.8	10	10	10

What this data indicates is that 100% of the data measured is greater than or equal to 1.0 microns. Ninety percent is greater that 1.4 microns diameter. This information when plotted on log-probability paper will yield a straight line if the distribution is truly log normal (most sample are). Once that data is plotted many statistical parameters are available to the analyst from standard formulas.

The second method of analysis is performed by a standard sieving technique. In this method, a known weight of dry sample is passed through nested precision sieves and the weight percent retained on each sieve size is calculated. Data is handled as above in Table I except data is expressed on a weight basis.

Since the Elzone technique determines the volume of individual particles, we can convert frequency data directly into mass or into area. It is part of the job of the technologist to determine which data format is appropriate to his application.

The Elzone data report is broken down as follows:

Page	Description
1	Frequency and Volume (Mass) Statistics
2	Plot of Differential Frequency Distribution
3	Tabulation of Channel Number, Diameter and Count (Number of particles at that size)
4	Plot of Differential Mass Distribution
5	Tabulation of Channel Number, Diameter and mass (relative units at that size)

Each page will be described below:

Page 1

The top section of this page is devoted to the volume (mass) statistics. The definitions of the terms used are as follows:

Volume Mode - The diameter size in microns of a spherical particle that contains the largest total mass value. It is always the peak of a distribution curve.

Volume Median -
That point in the distribution curve that splits the data into two equal areas. One half is larger and one half is smaller than the indicated size on a mass basis.

Geometric Volume Mean -
The size of an average particle calculated on a log basis.

Arithmetic Volume Mean -
The size of an average particle calculated on an arithmetic scale.

+/-XXX - One sigma interval of standard deviation

(XX.XXX) - Coefficient of variation. This is the Standard Deviation divided by the Mean mulitplied by 100 to yield percentage.

Skewness - This term denotes symmetry. If the curve is perfectly Gaussian, geometric skewness will be 0.00. If the curve is biased towards the fines, skewness will be negative.

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115 Hahn Street • Elmhurst, Illinois 60126 • (312) 832-5658

THE IMAGE ANALYZER EXPLANATION SHEET

The following explanation concerns the sample you have submitted or might want to submit for analysis by the Bausch and Lomb OMNICON 5000 Image Analyzer. It also, concerns how Particle Data Laboratories may best serve you in the field of image analysis.

The purpose of the image analyzer is to derive quantitative information from optical images automaticallly with the assistance of a computer. Besides counting and sizing to specifications of width, length, area and ten other measurement parameters, the image analyzer quantifies shape into factors of circularity and sphericity.

The Basis of Imaging and the Capabilities of the Image Analyzer

The interface between the optical image and the computer is a precision scanner which operates like a video camera to transform the microscopic image into electronic impulses. The image is displayed onto a black and white CRT monitor from which the analysis is conducted. Our image consists of various grey levels (object features) relative to a fixed background level. Therefore, the features in the image are defined by specific light level changes. The projected image is made up of picture points, the distance, of which, is precisely known through a calibration routine in any of four different magnification ranges.

Data is generated from a specific feature by summing the number of picture points in the entire feature or summing the linear distances between the picture points, (pixles). Variations on these two concepts allow the technology to develop the sixteen different shape measurements programmed into the instrument.

A single image or a portion of all the images present on the CRT can be anayzed. These may be a group of dispersed particles, the space between specific features or inclusions within specific features. The seven page insert describes in some detail each type of measurement used and/or available for our clients.

The OMNICON 5000 has a program mode that incorporates automatic stage movement and focus. The desired information is accumulated from multiple fields of analysis with a report generated from each field if desired. Usually, a single report is generated from the total accumulated data base.

For Plotting on Log Probability Paper -
This data is presented at 0.77 sigma intervals across a normal curve. It expresses the percent of mass at or greater than the indicated size from a cumulative curve.

The bottom of this page is just like the top except that it expresses the statistics on a frequency (count) basis.

Remember that the frequency basis will always be smaller than mass basis because the mass data rises as a function of the diameter cubed. It takes one million one micron diameter particles by count to equal the same mass as a single one hundred micron diameter particle.

Page 2

This page is a plot of the frequency distribution as a function of size. Each plus (+) represents a specific number of particles at a given size. The size scale is a log scale because a Gaussian curve plotted on a arithmetic scale would be skewed towards the larger sizes. Typically, data is plotted on a log scale.

Page 3

This is the "Tabulation" page by frequency (count). The number after "Total =" represents the number of particles counted in a particular analysis. This number is usually modified by some factor so that the graph routine will be represented as a full scale plot. The tabulation informs the client how may particles (count) he could expect to find at any indicated micron size if he had counted the number of particles indicated under "Total In Tabulation".

Page 4

This page is a plot of the mass (volume) distribution mathematically derived from the count (frequency) distribution. It reveals the distribution of mass as a function of particle size. Usually, this data is more relevant as to a particular industrial process.

Page 5

The last page in your report is a tabulation of data in a mass (volume) format. It is exactly like the count tabulation except that it informs us of the relative mass (grams, micrograms, pounds or tons) of material at each micron size if you had a pile of material weighing the same as that figure displayed under "Total =".

Once the data has been accumulated, it can be presented in any of three different formats: Linear, Logarithmic, or an Arbitrary Classification. In a linear distribution, the classes are all of the same size. Linear scales usually have between 8 and 25 class intervals. A log scale gives a better picture of the data when a large percentage of the features are either very small or very large. Arbitrary Classification distributions show in detail the way the measurements are distributed when they are clustered in a few classes on a linear scale. Following the selection of an output scale, the data can then be printed in each of the different measurement formats.

The ability of the analyzer to present data on several different formats requires us to obtain a very clear definition of the analytical problem from the client. It is usually necessary for us to ask many questions about samples requiring analysis by this technique.

An Explanation of the Computer Generated Output

A typical report is composed of three sections:

1. Statistics
2. Data Histogram
3. Class Information (Limits, Count, Percent)

A statistical summary indicating the type of distribution presented appears at the top of the page followed by a bar graph and a table of results. The statistical summary consists of 10 items which are listed below:

FEATURES: the total number images processed by the computer

FIELDS: the total number of microscopic fields of view evaluated

RANGE: the difference between the largest and the smallest feature measurement

MIN: the smallest feature measurement detected

MAX: the largest feature measurement detected

TOTAL: the sum of the products of the mid-points of the class sizes and "count"

MEAN: the TOTAL divided by the number of features

DEV: the standard deviation

HIGH: the total number of features detected greater than the upper ananalytical bound of the analysis

LOW: the total number of features detected greater than the lower analytical bound but not necessarily considered in the statistical data.

MDIAN: one half of the data is to the left of this size and one half of the data is to the right of this indicated size

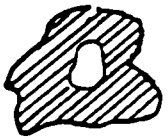
All distribution data is plotted as a histogram on either a differential or cumulative basis. The x-axis is the number of classes specified by the operator and the y-axis is the percentage of features whose measurements fall in each class.

The third section of the report is simply a tabulation of the selected class numbers, class interval sizes, feature count and percent on a differential or cumulative basis.

If there are any questions concerning this report format, please do not hesitate to contact us at Particle Data Laboratories.

In all of the diagrams below, the shaded area illustrates the measurement.

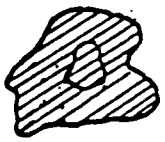
3.1. AREA MEASUREMENTS



AA - AREA

AREA is the area excluding holes, i.e. the area of a feature less the area of any holes, voids, or inclusions.

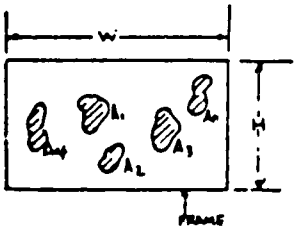
AREA gives the area of a cell less the area of the nucleus, or the area of a phase in a metal specimen less the area of the inclusions, and other similar area measurements.



AF - AREA FILLED

AREA FILLED is the area with holes filled, i.e. the area of a feature with the holes filled in.

AREA FILLED eliminates the effect of highlights in measuring the area of a convex reflecting object, and other similar holes which should not be measured.

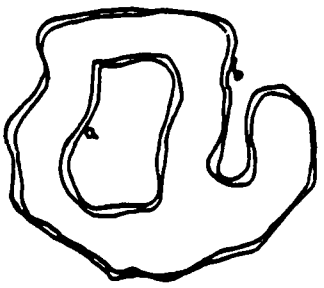


%A - PERCENT AREA

PERCENT AREA is the percentage of the field area occupied by detected features.

$$\%A = 100 \times \frac{\sum A_n}{W \times H}$$

3.2. PERIMETER MEASUREMENTS

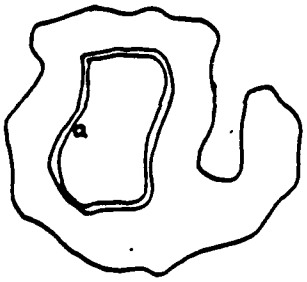


PERIMETER is the length of all the feature boundaries, including the interior boundaries.

PERIMETER gives the extent of coast line in an aerial photograph, or the dimensions of a cell wall, and other similar measurements.

PR - PERIMETER

PR = a + b

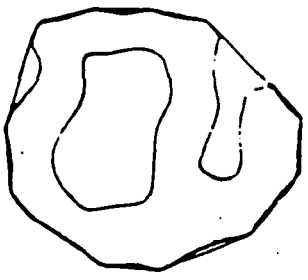


HOLE PERIMETER is the perimeter of holes, i.e. the perimeter of all the holes within a feature.

HOLE PERIMETER gives the measurement of boundaries of cell nuclei or inclusions, and other holes.

PH - HOLE PERIMETER

PH = a

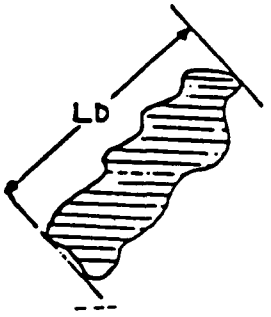


CONVEX PERIMETER is frequently called the rubber band or taut string measurement around a feature. It is the length of the minimum convex hull that can circumscribe a feature.

CONVEX PERIMETER eliminates the complexity of a feature boundary.

CP - CONVEX PERIMETER

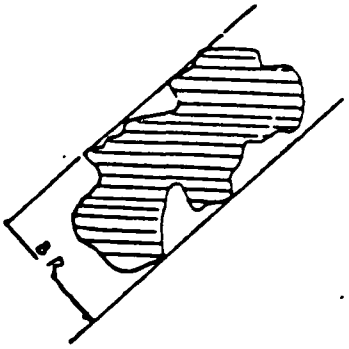
3.3. LINEAR AND COORDINATE MEASUREMENTS



LD - LONGEST DIMENSION

LONGEST DIMENSION is the maximum Feret measurement of a feature, based on eight Feret measurements, one every 22½° .

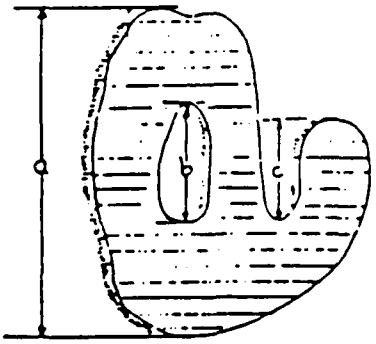
LONGEST DIMENSION measures lengths of inclusions in rolled steel, maximum lengths of particles in parenteral fluids, and other such lengths.



BR - BREADTH

BREADTH is the minimum Feret measurement of a feature, based on 16 Feret measurements, one every 11¼° .

BREADTH measures width, such as the width of fibers or printed lines or printed circuit boards.



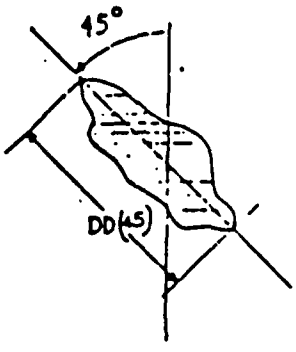
PL - PROJECTED LENGTH

PL = a + b + c

PROJECTED LENGTH is the tangent-to-tangent distance perpendicular to the scan lines on the leading edge of the feature and on the leading edges of any inclusions, or the sum of the distances between all scan line intercepts on the leading edge of the feature and any inclusions.

PROJECTED LENGTH gives three dimensional measurements from a two dimensional sample. Such measurements are called stereological.

PROJECTED LENGTH shows microstructures of materials like metals.

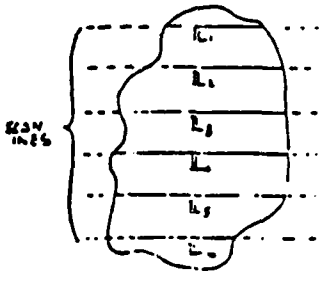


DIRECTED DIAMETER is Feret's diameter, the tangent-to-tangent distance at a specified point. The point is specified as an angle from a perpendicular dropped from the top of the monitor and called 0° . Counterclockwise is the positive direction.

The term diameter does not refer to any circle.

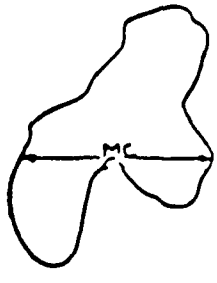
DIRECTED DIAMETER is useful in sizing particles.

DD - DIRECTED DIAMETER
DD(45)
A Directed Diameter of 45°



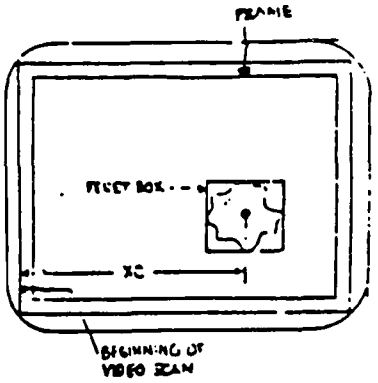
INTERCEPT LENGTH is given as a list of values. Each represents the length of an individual scan line chord in a feature.

IL - INTERCEPT LENGTH



MAXIMUM CHORD is the length of the maximum continuous horizontal intercept.

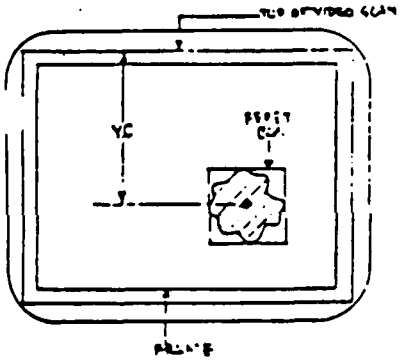
MC - MAXIMUM CHORD



XC - X COORDINATE

X COORDINATE is the horizontal position of the center of the Feret's box of a feature, taken from the beginning of the video scan at the left of the monitor screen.

X COORDINATE shows position and spatial distribution.



YC - Y COORDINATE

Y COORDINATE is the vertical position of the center of Feret's box of a feature, taken from the beginning of the video scan at the top of the monitor screen.

Y COORDINATE shows position and spatial distribution.

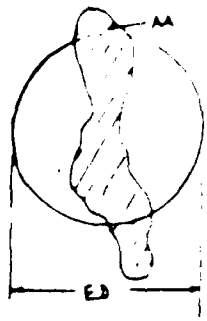
MI - MEAN INTERCEPT LENGTH

Appendix A

$$MI = \frac{A_1 + A_2 + A_3 + \ldots A_n}{PL_1 + PL_2 + PL_3 + \ldots PL_n}$$

MEAN INTERCEPT LENGTH IS TOTAL AREA divided by TOTAL PROJECTED LENGTH.

MEAN INTERCEPT LENGTH is used with the grain size tables of the American Society for Testing Materials, ASTM. It is also used to determine the mean free path, i.e. the average distance between features.

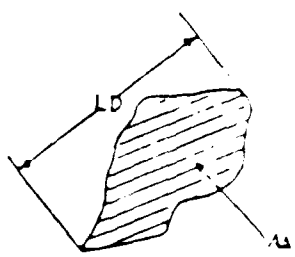


EQUIVALENT CIRCULAR DIAMETER is the diameter of a circle with the same area as the feature.

ED - EQUIVALENT CIRCULAR DIAMETER

$$ED = \sqrt{4(AA)/\pi}$$

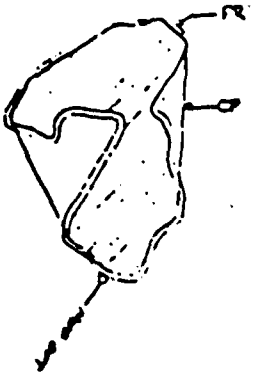
3.4. SHAPE FACTORS



CIRCULARITY is a shape factor which depends on the LONGEST DIMENSION of a feature and is relative to the area of a circle. CIRCULARITY is 1 for a circle, and its range is 1 - ∞.

CR - CIRCULARITY

$$CR = \pi (LD)^2 / 4(AA)$$



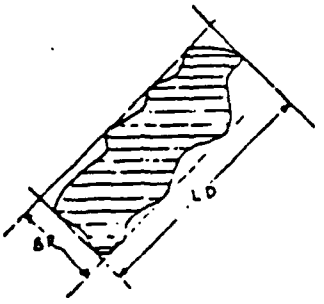
AGGLOMERATE REJECT is a shape factor which depends on boundary indentation or roughness of a feature.

The range is 1 for a circle to ∞.

When the AGGLOMERATE REJECT for a feature is larger than 1, the feature is suspected to be an agglomerate.

RO - AGGLOMERATE REJECT

RO = PR/CP



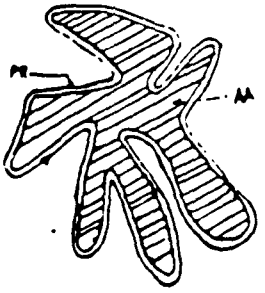
ELONGATION RATIO is a shape factor which depends on the LONGEST DIMENSION and BREADTH, but is insensitive to roughness.

The range is 1 for a circle to ∞.

Ideally no ELONGATION RATIO is less than 1, but for very small features it can be as small as 0.8.

EL - ELONGATION RATIO

EL = LD/BR



SPHERICITY is a shape factor depending on PERIMETER and AREA.

The range is 1 for a circle to zero.

SP - SPHERICITY

$SP = 4(AA)/ PR^2$

D-85-01
June 5, 1987

STANDARD OPERATING PROCEDURE
QUANTIFICATION OF SiO_2 IN COMPLEX MATRIX SAMPLES BY ICP OPTICAL EMISSION SPECTROSCOPY

Record all weights and data in the laboratory record book. All chemicals must be reagent grade or better.

1. SAMPLE PREPARATION

   A. Prepare standards in duplicate by spiking a 0.1 g aliquot of matrix or simulated matrix material with SiO_2 so that the final concentration of SiO_2 is in the expected range of samples.

   B. Mix samples (~0.1g dry wt.) and spiked standards with 2g Na_2SO_3 and 1g $Na_2B_4O_7$. Fuse these samples and standards in platinum crucibles or equivalent by placing in a muffle furnace at 1000°C for 30 minutes.

   C. Leach the fused samples & standards with 25 ml of 20% HCl.

   D. Dilute the leached samples and standards to 100 ml with 20% HCl.

   E. Prepare a matrix blank by treating a 0.1 g aliquot of unspiked matrix material as in steps 1-A thru D above.

2. SAMPLE ANALYSIS

   Determine the Si content of the samples and standards by ICP and express in ppm.

3. CALCULATIONS

   A. Convert ppm Si to mg SiO_2 using the following equation:

   $$\text{mg } SiO_2 = \frac{\text{ppm}}{10} \times 2.139$$

   $$\text{where } 2.139 = \frac{\text{m. wt. } SiO_2}{\text{atom wt. Si}}$$

   B. Calculate % SiO_2 as follows:

   $$\% \ SiO_2 = \frac{\text{mg } SiO_2}{\text{Sple.wt. (mg)}} \times 100$$

   C. Determine % recovery from the spiked standards as follows:

   $$\% \text{ Recovery} = \frac{\text{mg } SiO_2 \text{ found (Step 3.A.)}}{\text{mg } SiO_2 \text{ added (Step 1.A.)}} \times 100\%$$

Appendix A

APPROVALS:

C. Faber 6-9-87
C. M. Faber Date
Researcher

Ming J.W. Chang 6-10-87
M.J.W. Chang, Ph.D. Date
Chemistry Discipline Leader
Toxicology & Health Sciences Section

G.L. Fisher, Ph.D. Date
Acting Manager
Toxicology & Health Sciences Section

G.L. Fisher, Ph.D. Date
Vice President
Toxicology & Pharmacology Department

Ramona A. Mayer, Manager Date
Quality Assurance Unit

Anna D. Barker, Ph.D. 6/19/87 Date
Senior Vice President
Biological & Chemical Sciences

Blank

APPENDIX B

INCIDENCE AND SEVERITY SUMMARIES OF MICROSCOPIC FINDINGS FROM RANGE-FINDING AND DEFINITIVE STUDIES

Blank

Appendix B

INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
RANGE-FINDING STUDY

Observation/Severity		Media Control
N[a]		15[b]/27[c]
Normal		7/12
Cuboidal - squamous	**Total[d] =**	**4/21**
change	1	3/13
	2	1/8
M[illegible] degeneration/ [illegible]s	**Total =**	**6/4**
	1	5/4
	2	1/-
M[illegible] hyperplasia	**Total =**	**4/1**
	1	3/1
	2	1/-

[a] The total number of tracheal explants examined.

[b] The first column lists the number of explants collected and examined 1 week following exposure listing the total number with the indicated finding and severity.

[c] The second column lists the number of explants collected and examined 3 weeks following exposure listing the total number with the indicated finding and severity.

[d] Total - Total number of explants with indicated lesions. 1 = mild, 2 = moderate, 3 = severe.

INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
RANGE-FINDING STUDY

Glass Beads

Observation/Severity		Dose Level 1 µg/ml	10 µg/ml	100 µg/ml	1 mg/ml	10 mg/ml	100 mg/ml
N[a]		8[b]/10[c]	10/10	9/9	10/10	10/10	8/5
Normal		7/6	6/5	6/4	9/5	8/5	6/3
Epidermoidal - squamous	**Total[d] =**	**1/-**	**1/3**	**1/3**	**4/2**	**1/-**	**-/1**
change	1	1/-	1/3	1/3	4/2	1/-	-/1
Mucosal degeneration/ necrosis	**Total =**	**-/3**	**1/2**	**2/2**	**-/4**	**-/4**	
	1	-/3	1/2	2/2	-/3	-/3	
	2					-/1	
Mucosal hyperplasia	**Total =**	**-/2**	**2/1**		**1/-**	**1/-**	**2/1**
	1	-/2	2/-		1/-	-/1	1/1
	2		-/1				1
Squamous metaplasia	**Total =**			**-/1**			
	1			-/1			

[a]N = The total number of tracheal explants examined.

[b]Left column lists the number of explants collected and examined 1 week following exposure listing the total number with the indicated finding and severity.

[c]Right column lists the number of explants collected and examined 3 weeks following exposure listing the total number with the indicated finding and severity.

[d]Total = Total number of explants with indicated lesions. 1 = mild, 2 = moderate, 3 = severe.

Appendix B

INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
RANGE-FINDING STUDY

Crocidolite Asbestos

Observation/Severity		Dose Level 1 µg/ml	10 µg/ml	100 µg/ml	1 mg/ml	10 mg/ml	100 mg/ml
N[a]		9[b]/10[c]	9/10	9/11	10/10	11/10	9/8
Normal		5/7	1/6	3/3	2/5	2/2	2/-
Cuboidal - squamous change	Total[d] =	1/-	-/1	5/3	-/4	4/3	5/-
	1	1/-	-/1	2/2	-/3	2/2	3/-
	2			1/-	-/1	2/-	2/-
	3			2/1		-/1	
Mucosal degeneration	Total =	-/1	3/3	3/5	7/1	2/7	5/7
	1	-/1	3/2	3/4	6/1	2/5	3/4
	2		-/1	-/1	1/-	-/2	2/2
	3						-/1
Mucosal hyperplasia	Total =	1/-	4/-		3/-	7/2	1/-
	1	-/-	1/-		2/-	6/2	1/-
	2	1/-	2/-		-/-	1/-	
	3		1/-		-/-		
Squamous metaplasia	Total =		2/-		-/1	-/3	-/4
	1		2/-		-/1	-/3	-/4
Basal cell hypertrophy and hyperplasia	Total =	1/2	-/1	2/-	-/1		
	1	1/2	-/1	2/-	-/1		
	2				-/-		
	3				1/-		
Mucosal dysplasia	Total =	1/-					
	1	1/-					

[a]N = The total number of tracheal explants examined.

[b]Left column lists the number of explants collected and examined 1 week following exposure listing the total number with the indicated finding and severity.

[c]Right column lists the number of explants collected and examined 3 weeks following exposure listing the total number with the indicated finding and severity.

[d]Total = Total number of explants with indicated lesions. 1 = mild, 2 = moderate, 3 = severe.

INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
RANGE-FINDING STUDY

Graphite Fibers

Observation/Severity		Dose Level 1 µg/ml	10 µg/ml	100 µg/ml	1 mg/ml	10 mg/ml	100 mg/ml
N[a]		9[b]/10[c]	10/10	9/11	8/10	10/10	10/10
Normal		4/2	2/4	2/3	8/2	4/-	-/-
Cuboidal - squamous change	**Total[d] =**	**3/6**	**6/2**	**3/6**	**-/2**	**6/4**	**8/9**
	1	2/5	6/2	2/3	-/-	3/2	3/7
	2	1/1		1/3	-/2	1/1	3/-
	3					2/1	2/2
Mucosal degeneration/ necrosis	**Total =**	**-/2**	**2/6**	**5/3**	**-/7**	**-/4**	**1/6**
	1	-/2	2/3	4/3	-/6	-/2	1/2
	2		-/3	1/-	-/1	-/1	1/3
	3					-/1	1/1
Mucosal hyperplasia	**Total =**	**2/2**	**1/2**	**-/2**	**-/2**	**-/1**	**3/1**
	1	2/2	1/2	-/2	-/2	-/-	2/1
	2					-/1	1/-
Squamous metaplasia	**Total =**					**-/2**	**3/4**
	1					-/2	3/3
	2						-/1
Basal cell hypertrophy	**Total =**					**-/2**	
	1					-/1	
	2					-/1	

[a] N = The total number of tracheal explants examined.

[b] Left column lists the number of explants collected and examined 1 week following exposure listing the total number with the indicated finding and severity.

[c] Right column lists the number of explants collected and examined 3 weeks following exposure listing the total number with the indicated finding and severity.

[d] Total = Total number of explants with indicated lesions. 1 = mild, 2 = moderate, 3 = severe.

INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
RANGE-FINDING STUDY

KS-2 Graphite

Observation/Severity		Dose Level 1 µg/ml	10 µg/ml	100 µg/ml	1 mg/ml	10 mg/ml	100 mg/ml
N[a]		7[b]/10[c]	9/10	9/10	10/10	11/10	12/10
Normal		4/2	3/1	2/-	-/-	1/-	1/-
Cuboidal - squamous change	**Total[d] =**	**3/8**	**4/6**	**4/8**	**4/7**	**10/8**	**10/10**
	1	2/7	3/4	3/6	3/7	7/6	4/4
	2	1/1	1/2	1/1	1/-	3/2	5/6
	3			-/1			1/-
Mucosal degeneration/ necrosis	**Total =**	**-/3**	**-/5**	**2/5**	**5/6**	**3/1**	**2/3**
	1	-/3	-/5	1/3	5/5	3/1	2/3
	2			1/2	-/1		
Mucosal hyperplasia	**Total =**	**1/-**	**2/3**	**2/1**	**1/3**	**2/-**	**4/-**
	1	1/-	1/2	1/1	1/2	2/-	4/-
	2		1/1	-/-	-/1		
	3			1/-			
Squamous metaplasia	**Total =**	**-/3**	**2/-**		**1/1**	**5/-**	
	1	-/3	2/-		1/1	5/-	
Mucosal dysplasia	**Total =**	**-/1**		**2/3**	**2/2**	**1/3**	**3/-**
	1	-/1		-/2	2/2	1/3	1/-
	2			2/1			2/-

[a]N = The total number of tracheal explants examined.

[b]Left column lists the number of explants collected and examined 1 week following exposure listing the total number with the indicated finding and severity.

[c]Right column lists the number of explants collected and examined 3 weeks following exposure listing the total number with the indicated finding and severity.

[d]Total = Total number of explants with indicated lesions. 1 = mild, 2 = moderate, 3 = severe.

INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
RANGE-FINDING STUDY

Micro - 260 Graphite

Observation/Severity		Dose Level 1 µg/ml	10 µg/ml	100 µg/ml	1 mg/ml	10 mg/ml	100 mg/ml
N[a]		8[b]/10[c]	10/10	10/10	10/10	9/10	10/10
Normal		1/-	2/2	1/-	3/-	2/2	2/-
Cuboidal - squamous change	**Total[d] =**	**6/9**	**3/8**	**4/8**	**4/7**	**5/8**	**6/9**
	1	2/7	1/6	3/8	4/5	3/4	3/5
	2	4/2	2/2	1/-	-/2	1/3	3/4
	3					1/1	
Mucosal degeneration/ necrosis	**Total =**	**-/4**	**2/-**	**4/4**	**6/1**	**3/-**	**8/3**
	1	-/4	2/-	4/4	6/-	2/-	7/3
	2				-/1	1/-	1/-
Mucosal hyperplasia	**Total =**	**1/-**	**4/-**	**1/-**	**-/3**	**3/-**	**1/-**
	1	1/-	2/-	-/-	-/1	1/-	1/-
	2		2/-	1/-	-/2	2/-	
Squamous metaplasia	**Total =**			**1/-**		**-/1**	
	1			1/-		-/1	
Mucosal dysplasia	**Total =**	**-/2**	**-/2**	**-/5**	**-/4**	**1/5**	**2/2**
	1	-/2	-/2	-/5	-/2	1/5	1/2
	2				-/2		1/-
Basal cell hypertrophy	**Total =**			**4/-**			
	1			1/-			
	2			3/-			

[a] N = The total number of tracheal explants examined.

[b] Left numeral lists the number of explants collected and examined 1 week following exposure listing the total number with the indicated finding and severity.

[c] Right numeral lists the number of explants collected and examined 3 weeks following exposure listing the total number with the indicated finding and severity.

[d] Total = Total number of explants with indicated lesions. 1 = mild, 2 = moderate, 3 = severe.

INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
RANGE-FINDING STUDY

Printex L-Carbon Black

Observation/Severity		Dose Level 1 µg/ml	10 µg/ml	100 µg/ml	1 mg/ml	10 mg/ml	100 mg/ml
N[a]		9[b]/10[c]	9/10	10/10	9/10	8/10	9/9
Normal		4/1	4/3	4/2	1/-	3/2	1/1
Cuboidal - squamous change	**Total[d] =**	**5/4**	**4/3**	**3/2**	**7/7**	**4/4**	**7/6**
	1	4/4	1/2	1/1	3/6	3/3	1/4
	2	1/-	3/1	2/1	3/1	1/1	3/1
	3				1/-		3/1
Mucosal degeneration/ necrosis	**Total =**	**-/6**	**1/1**	**5/4**	**4/6**	**1/4**	**5/4**
	1	-/5	-/1	5/1	3/3	-/1	5/4
	2	-/1	1/-	-/3	1/3	1/2	
	3					-/1	
Mucosal hyperplasia	**Total =**		**1/2**	**-/1**		**1/2**	**1/-**
	1		1/1	-/1		-/1	1/-
	2		-/1			1/1	
Squamous metaplasia	**Total =**				**1/1**		**-/1**
	1				1/1		-/1
Mucosal dysplasia	**Total =**		**-/3**	**-/6**	**-/1**	**1/2**	**-/5**
	1		-/2	-/5	-/1	-/1	-/4
	2		-/1	-/1		-/1	-/1
	3					1/-	

[a]N = The total number of tracheal explants examined.

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[c]Right column lists the number of explants collected and examined 3 weeks following exposure listing the total number with the indicated finding and severity.

[d]Total = Total number of explants with indicated lesions. 1 = mild, 2 = moderate, 3 = severe.

INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
RANGE-FINDING STUDY

Aluminum Dust

Observation/Severity		Dose Level 1 µg/ml	10 µg/ml	100 µg/ml	1 mg/ml	10 mg/ml	100 mg/ml
N[a]		10[b]/10[c]	9/10	9/10	9/10	9/10	10/10
Normal		5/3	5/6	1/2	3/2	3/5	2/1
Cuboidal - squamous change	**Total[d] =**	**2/4**	**2/1**	**3/3**	**4/4**	**5/2**	**3/5**
	1	2/2	2/-	2/2	2/1	3/1	3/2
	2	-/2	-/1	1/-	1/2	2/1	-/3
	3			-/1	1/1		
Mucosal degeneration/ necrosis	**Total -**	**4/4**	**-/3**	**4/4**	**3/4**	**2/1**	**3/6**
	1	2/2	-/3	4/2	2/3	2/-	3/2
	2	2/2		-/2	1/1	-/1	-/1
Mucosal hyperplasia	**Total =**	**1/1**		**4/1**	**2/-**	**2/5**	**5/4**
	1	1/1		4/1	1/-	1/4	2/4
	2				1/-	1/1	3/-
Squamous metaplasia	**Total =**		**1/-**				
	1		1/-				
Basal cell hypertrophy and hyperplasia	**Total =**		**2/-**				
	1		2/-				

[a]N = The total number of tracheal explants examined.

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INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
RANGE-FINDING STUDY

Polycrystalline Iron Whiskers

Observation/Severity		Dose Level 1 µg/ml	10 µg/ml	100 µg/ml	1 mg/ml	10 mg/ml	100 mg/ml
N[a]		9[b]/10[c]	9/10	10/10	11/9	9/9	9/10
Normal		2/3	4/2	5/2	4/2	1/-	
Cuboidal - squamous change	**Total[d] =**	**4/5**	**3/5**	**4/2**	**5/6**	**6/7**	**4/-**
	1	2/4	2/2	2/1	4/2	3/6	-/-
	2	2/1	-/2	1/1	1/4	3/1	1/-
	3		1/1	1/-			3/-
Mucosal degeneration/ necrosis	**Total =**	**6/4**	**1/5**	**1/6**	**2/3**	**3/5**	**8/10**
	1	5/4	1/3	1/4	1/3	3/4	1/-
	2	1/-	-/2	-/2	1/-	-/1	2/-
	3						5/10
Squamous metaplasia	**Total =**	**-/1**	**-/2**	**-/2**	**-/3**	**1/5**	
	1	-/1	-/2	-/2	-/3	1/4	
	2					-/1	
Basal cell hypertrophy & mucosal hyperplasia	**Total =**	**2/4**	**1/1**	**-/2**	**1/-**		
	1	1/3	-/1	-/2	-/-		
	2	1/1	1/-		-/-		
	3				1/-		
Basal cell hypertrophy	**Total =**					**-/1**	
	1					-/-	
	2					-/1	

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[b]Left column lists the number of explants collected and examined 1 week following exposure listing the total number with the indicated finding and severity.

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Appendix B

INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
RANGE-FINDING STUDY

Brass Dust

Observation/Severity		Dose Level 1 µg/ml	10 µg/ml	100 µg/ml	1 mg/ml	10 mg/ml	100 mg/ml
N[a]		9[b]/10[c]	7/10	10/10	10/9	10/10	9/10
Normal		4/4	1/-	4/1			
Cuboidal - squamous	Total[d] =	3/4	2/7	5/7	8/4		
change	1	-/2	-/5	2/3	-/-		
	2	2/2	1/2	3/4	3/1		
	3	1/-	1/-		5/3		
Mucosal degeneration/ necrosis	Total =	1/4	3/5	-/3	10/9	10/10	9/10
	1	1/2	2/3	-/3	5/-	-/-	-/-
	2	-/2	-/1		3/4	-/-	5/5
	3		1/1		2/5	10/10	4/5
Mucosal hyperplasia	Total	1/-	3/3	3/2	-/1		
	1	-/-	1/1	1/1	-/1		
	2	1/-	2/2	2/1			
Squamous metaplasia	Total		1/1				
	1		-/1				
	2		1/-				
Mucosal dysplasia	Total =	1/-	1/3	3/3			
	1	-/-	-/1	3/2			
	2	1/-	1/2	-/1			

[a] N = The total number of tracheal explants examined.

[b] Left column lists the number of explants collected and examined 1 week following exposure listing the total number with the indicated finding and severity.

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INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
RANGE-FINDING STUDY

Ni-Coated Graphite

Observation/Severity		Dose Level 1 µg/ml	10 µg/ml	100 µg/ml	1 mg/ml	10 mg/ml	100 mg/ml
N[a]		8[b]/10[c]	10/10	10/10	10/9	10/8	10/9
Normal		1/-	1/1		2/-		
Cuboidal - squamous change	**Total[d] =**	**5/10**	**4/7**	**8/7**	**7/8**	**7/1**	
	1	2/8	2/6	7/6	1/4	1/-	
	2	3/1	2/1	1/1	2/3	1/-	
	3	-/1			4/1	5/1	
Mucosal degeneration/ necrosis	**Total =**	**6/5**	**2/6**	**4/6**	**5/2**	**9/8**	**10/9**
	1	5/3	2/5	3/4	3/1	2/1	-/-
	2	1/2	-/1	1/2	2/1	4/-	-/-
	3					3/7	10/9
Mucosal hyperplasia	**Total =**	**2/1**	**4/-**		**1/-**		
	1	2/1	1/-		1/-		
	2		2/-				
	3		1/-				
Squamous metaplasia	**Total =**		**1/1**	**1/-**	**1/2**	**3/-**	
	1		1/1	1/-	1/2	3/-	
Muscosal dysplasia	**Total =**			**-/1**	**-/2**	**1/-**	
	1			-/1	-/1	-/-	
	2				-/1	1/-	

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[b]Left column lists the number of explants collected and examined 1 week following exposure listing the total number with the indicated finding and severity.

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INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
DEFINITIVE STUDY

Media Control

Observation		
N[a]		25[b]/15[c]
Normal		15/4
Cuboidal - squamous change	**Total[d] =**	**7/5**
	1	4/5
	2	3/-
Mucosal degeneration/ necrosis	**Total =**	**-/2**
	1	-/1
	2	-/1
Mucosal hyperplasia	**Total =**	**-/-**
Squamous metaplasia	**Total =**	**1/4**
	1	1/3
	2	-/1
Mucosal dysplasia	**Total =**	**1/-**
	1	-/-
	2	1/-

[a]N = The total number of tracheal explants examined.

[b]Left column lists the number of explants collected and examined 1 week following exposure listing the total number with the indicated finding and severity.

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INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
DEFINITIVE STUDY

Glass Beads

Observation/Severity		Dose Level 100 µg/ml	1 mg/ml	10 mg/ml
N[a]		13[b]/14[c]	14/15	15/14
Normal		9/8	2/2	8/2
Cuboidal - squamous change	**Total[d] =**	**2/4**	**12/11**	**2/1**
	1	2/4	8/4	2/1
	2		4/7	
Mucosal degeneration/ necrosis	**Total =**	**2/2**	**2/5**	**5/4**
	1	2/2	1/5	5/4
	2		1/-	
Mucosal hyperplasia	**Total =**		**-/4**	**3/6**
	1		-/4	1/5
	2			2/1
Squamous metaplasia	**Total =**	**-/1**	**-/2**	**-/3**
	1	-/1	-/2	-/3
Mucosal dysplasia	**Total =**	**-/1**	**-/1**	**-/4**
	1	-/1	-/1	-/1
	2			-/3

[a]N = The total number of tracheal explants examined.

[b]Left column lists the number of explants collected and examined 1 week following exposure listing the total number with the indicated finding and severity.

[c]Right column lists the number of explants collected and examined 3 weeks following exposure listing the total number with the indicated finding and sevrity.

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INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
DEFINITIVE STUDY

Crocidolite Asbestos

Observation/Severity		Dose Level 100 µg/ml	1 mg/ml	10 mg/ml
N[a]		15[b]/14[c]	14/15	16/14
Normal		7/1	5/2	3/1
Cuboidal - squamous change	Total[d] =	**8/5**	**-/2**	**8/1**
	1	5/3	-/1	5/1
	2	3/2	-/1	3/-
Mucosal degeneration/	Total =	**-/3**	**1/1**	**8/3**
	1	-/3	1/1	5/3
Mucosal hyperplasia	Total =	**2/5**	**5/6**	**5/3**
	1	2/5	3/3	4/3
	2		1/3	1/-
	3		1/-	
Squamous metaplasia	Total =	**-/6**	**1/9**	**-/8**
	1	-/5	1/8	-/7
	2	-/1	-/1	-/1
Mucosal dysplasia	Total =	**1/4**	**4/2**	**2/2**
	1	1/2	1/-	2/2
	2	-/2	2/1	
	3		1/1	

[a]N = The total number of tracheal explants examined.

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Appendix B

INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
DEFINITIVE STUDY

Graphite Fibers

Observation/Severity		Dose Level 100 µg/ml	1 mg/ml	10 mg/ml
N[a]		7[b]/0[c]	15/14	15/13
Normal		2/-	4/2	4/-
Cuboidal - squamous change	**Total[d] =**	**4/-**	**2/6**	**9/12**
	1	-/-	2/4	5/6
	2	2/-	-/2	4/5
	3	2/-		-/1
Mucosal degeneration/necrosis	**Total =**	**1/-**	**4/3**	**4/7**
	1	1/-	4/2	1/5
	2		-/1	3/2
Mucosal hyperplasia	**Total =**	**1/-**	**3/4**	**2/2**
	1	1/-	2/2	1/2
	2		1/2	1/2
Squamous metaplasia	**Total =**		**-/1**	**-/4**
	1		-/1	-/4
Mucosal dysplasia	**Total =**		**5/3**	**2/3**
	1		4/2	1/3
	2		1/1	1/-

[a]N - The total number of tracheal explants examined.

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INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
DEFINITIVE STUDY

KS-2 Graphite

Observation/Severity		Dose Level 100 µg/ml	1 mg/ml	10 mg/ml
N[a]		14[b]/15[c]	15/15	15/15
Normal		4/1	6/2	5/-
Epithelial - squamous	Total[d] =	**3/8**	**2/11**	**10/11**
change	1	2/5	2/6	7/5
	2	1/3	-/5	3/5
Mucosal hyperkeratosis	Total =	**4/1**	**7/1**	**4/1**
	1	4/1	7/1	3/-
	2			1/1
Mucosal hyperplasia	Total =	**5/2**	**1/2**	**1/2**
	1	3/2	1/2	1/2
	2	2/-	-/1	
Squamous metaplasia	Total =	**-/4**	**-/9**	**1/5**
	1	-/4	-/7	1/5
	2		-/1	
	3		-/1	
Mucosal dysplasia	Total =	**-/2**	**-/4**	**-/2**
	1	-/1	-/2	-/2
	2	-/1	-/1	
	3		-/1	

[a]N = The total number of tracheal explants examined.

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INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
DEFINITIVE STUDY

Micro-260 Graphite

Observation/Severity		Dose Level 100 µg/ml	1 mg/ml	10 mg/ml
N[a]		15[b]/14[c]	16/15	15/15
Normal		1/-	2/-	4/4
Cuboidal - squamous change	**Total[d] =**	**9/14**	**7/7**	**6/8**
	1	4/-	3/4	5/5
	2	5/12	2/3	1/3
	3	-/2	2/-	
Mucosal degeneration/ necrosis	**Total =**	**5/-**	**8/6**	**7/5**
	1	4/-	7/6	6/5
	2	1/-	1/-	1/-
Mucosal hyperplasia	**Total =**	**-/1**	**2/9**	**-1/1**
	1	-/1	1/9	-1/1
	2		-/-	
	3		1/-	
Squamous metaplasia	**Total =**	**4/3**	**-/2**	**1/4**
	1	3/3	-/2	1/4
	2	1/-		
Mucosal dysplasia	**Total =**	**2/-**	**1/-**	
	1	2/-	-/-	
	2		1/-	

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INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
DEFINITIVE STUDY

Printex L-Carbon Black

Observation/Severity		Dose Level 100 µg/ml	1 mg/ml	10 mg/ml
N[a]		15[b]/13[c]	13/14	15/15
Normal		2/1	1/-	3/-
Cuboidal - squamous change	Total[d] =	8/8	9/10	7/15
	1	5/7	6/6	5/8
	2	2/1	2/4	1/6
	3	1/-	1/-	1/1
Mucosal degeneration/ necrosis	**Total =**	**7/4**	**5/1**	**9/3**
	1	7/3	4/-	8/3
	2	-/1	1/1	1/-
Mucosal hyperplasia	**Total =**	**2/1**	**2/4**	
	1	1/-	1/2	
	2	1/1	1/2	
Squamous metaplasia	**Total =**	**3/8**	**-/8**	**1/2**
	1	2/8	-/8	-/2
	2	-/-		-/-
	3	1/-		
Mucosal dysplasia	**Total =**			**1/-**
	1			1/-

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INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
DEFINITIVE STUDY

Aluminum Dust

Observation/Severity		Dose Level 100 µg/ml	1 mg/ml	10 mg/ml
N[a]		14[b]/13[c]	15/13	15/15
Normal		-/2	2/-	-/2
Cuboidal - squamous change	**Total[d] =**	**9/4**	**8/5**	**15/11**
	1	4/2	7/3	4/4
	2	3/2	1/2	11/6
	3	2/-		-/1
Mucosal degeneration/ necrosis	**Total =**	**4/-**	**6/-**	**5/1**
	1	4/-	5/-	3/1
	2		1/-	2/-
Mucosal hyperplasia	**Total =**	**3/1**	**1/3**	**3/2**
	1	1/1	-/2	3/2
	2	2/-	1/1	
Squamous metaplasia	**Total =**	**-/3**	**-/5**	**-/5**
	1	-/3	-/4	-/5
	2		-/1	
Mucosal dysplasia	**Total =**	**4/8**	**6/6**	**-/1**
	1	4/6	4/5	-/-
	2	-/2	2/1	-/1

[a]N - The total number of tracheal explants examined.

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INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
DEFINITIVE STUDY

Polycrystalline Iron Whiskers

Observation/Severity		Dose Level 100 µg/ml	1 mg/ml	10 mg/ml
N[a]		14[b]/9[c]	14/15	15/15
Normal		2/-	4/1	1/2
Cuboidal - squamous change	**Total[d] =**	**11/-**	**6/1**	**11/2**
	1	9/-	1/1	5/2
	2	2/-	3/-	6/-
	3		2/-	
Mucosal degeneration/ necrosis	**Total =**	**4/9**	**6/2**	**7/-**
	1	3/2	4/1	6/-
	2	1/3	2/-	1/-
	3	-/4	-/1	
Mucosal hyperplasia	**Total =**		**1/6**	**3/8**
	1		-/5	2/3
	2		1/1	-/-
	3			1/-
Squamous metaplasia	**Total =**		**-/9**	**-/9**
	1		-/7	-/9
	2		-/2	
Mucosal dysplasia	**Total =**		**1/5**	**3/3**
	1		-/4	2/3
	2		1/1	1/-

[a] N - The total number of tracheal explants examined.

[b] Left column lists the number of explants collected and examined 1 week following exposure listing the total number with the indicated finding and severity.

[c] Right column lists the number of explants collected and examined 3 weeks following exposure listing the total number with the indicated finding and severity.

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INCIDENCE SUMMARY OF MICROSCOPIC FINDINGS
DEFINITIVE STUDY

Brass Dust

Observation/Severity		Dose Level 1 µg/ml	10 µg/ml	100 µg/ml	1 mg/ml
N[a]		15[b]/10[c]	12/2	12/14	15/14
Normal		5/-	3/-	-/-	-/-
Subnasal - squamous change	**Total[d] =**	**5/2**	**6/-**	**10/10**	**3/-**
	1	3/2	1/-	3/-	2/-
	2	2/-	4/-	5/2	1/-
	3		1/	2/8	
Mucosal degeneration/ necrosis	***Total*** **=**	**6/10**	**2/2**	**5/9**	**15/14**
	1	5/2	2/1	3/2	-/-
	2	1/6	-/1	-/3	3/-
	3	-/2		2/4	12/14
Mucosal hyperplasia	**Total =**		**-/1**	**-/2**	
	1		-/-	-/1	
	2		-/1	-/1	
Squamous metaplasia	**Total =**	**-/4**	**1/-**	**-/5**	
	1	-/3	-/-	-/3	
	2	-/1	1/-	-/2	
Mucosal dysplasia	**Total =**	**2/-**	**3/-**	**2/2**	
	1	2/-	2/-	2/1	
	2		1/-	-/1	

[a]N = The total number of tracheal explants examined.

[b]Left column lists the number of explants collected and examined 1 week following exposure listing the total number with the indicated finding and severity.

[c]Right column lists the number of explants collected and examined 3 weeks following exposure listing the total number with the indicated finding and severity.

[d]Total = Total number of explants with indicate lesions. 1 = mild, 2 = moderate, 3 = severe.

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APPENDIX C

INDIVIDUAL EXPLANT MORPHOMETRIC DATA AND STATISTICAL SUMMARIES

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KEY TO TEST ARTICLES

1 - Media Control

2 - Glass Beads

3 - Asbestos

4 - Iron Whiskers

5 - Graphite Fibers

6 - Aluminum Dust

7 - KS-2 Graphite

8 - Micro-260 Graphite

9 - Printex-L - Carbon Black

10 - Nickel Coated Graphite

11 - Brass Dust

100 UG/ML LEVEL

PERCENT MUCOSAL LESION - INDIVIDUAL EXPLANTS

TEST ARTICLE	PERCENT LESION	TEST ARTICLE	PERCENT LESION	TEST ARTICLE	PERCENT LESION
1	0.0	4	0.0	9	0.0
1	0.0	4	0.0	9	0.0
1	22.6	4	0.0	9	34.5
1	0.0	4	0.0	9	21.1
1	3.5	4	0.0	9	0.0
1	10.9	4	0.0	9	7.7
1	23.6	4	0.0	9	12.4
1	10.5	4	0.0	9	15.1
1	6.1			9	19.6
1	0.0	6	0.0	9	24.8
1	0.0	6	9.0	9	2.1
1	17.4	6	14	9	34.3
1	0.0	6	14.1	9	6.6
1	14.6	6	18.1		
1	41.5	6	2.7	10	0.0
		6	39	10	0.0
2	0.0	6	21.1	10	9.8
2	16	6	43.3	10	37.9
2	0.0	6	0.0	10	67.2
2	0.0	6	0.0	10	74
2	11.2	6	28.8	10	41.3
2	15.5	6	29.3	10	32.3
2	27			10	14.5
2	0.0	7	36.8	10	20.9
2	0.0	7	15.3	10	33.3
2	21.3	7	63.9	10	40.2
2	0.0	7	0.0	10	85.1
2	0.0	7	0.0		
2	8.9	7	26.4	11	58.3
2	0.0	7	0.0	11	57.7
		7	0.0	11	0.0
3	30.5	7	0.0	11	47.5
3	31.2	7	22.2	11	0.0
3	46.5	7	0.0	11	25.6
3	40	7	20.2	11	0.0
3	4.3	7	0.0	11	51.5
3	26.5	7	8.1	11	0.0
3	0.0	7	0.0	11	43.2
3	0.0			11	0.0
3	29	8	41.1	11	0.0
3	36.6	8	0.0	11	0.0
3	11.3	8	37.6	11	46.1
3	58.6	8	1.2		
3	94	8	0.0		
3	0.0	8	10.5		
3	5.2	8	0.0		
		8	33		
		8	12.6		
		8	0.0		
		8	0.0		
		8	0.0		
		8	0.0		
		8	19.3		

1 MG/ML LEVEL
PERCENT MUCOSAL LESION - INDIVIDUAL EXPLANTS

TEST ARTICLE	PERCENT LESION	TEST ARTICLE	PERCENT LESION	TEST ARTICLE	PERCENT LESION	TEST ARTICLE	PERCENT LESION
1	0.0	4	50.6	7	14.5	10	0.0
1	0.0	4	12.5	7	17.4	10	0.0
1	22.6	4	16.4	7	68.1	10	0.0
1	0.0	4	13	7	25.5	10	0.0
1	3.5	4	38.1	7	20.6	10	0.0
1	10.9	4	65.8	7	71.3	10	36.7
1	23.6	4	10.9	7	0.0	10	61.4
1	10.5	4	16.1	7	0.0	10	65.3
1	6.1	4	12.6	7	85.6	10	45.4
1	0.0	4	23.6	7	0.0	10	0.0
1	0.0	4	73.9	7	0.0	10	0.0
1	17.4	4	41.5	7	34.8	10	0.0
1	0.0	4	35.2	7	24.1	10	0.0
1	14.6	4	15.7	7	8.2	10	0.0
1	41.5			7	16.3		
		5	43.4			11	0.0
2	0.0	5	0.0	8	0.0	11	0.0
2	28.6	5	34.8	8	0.0	11	0.0
2	5.2	5	70.3	8	32.7	11	0.0
2	0.0	5	19.9	8	30.6	11	0.0
2	22.7	5	68.7	8	0.0	11	0.0
2	0.0	5	0.0	8	19.2	11	0.0
2	29.5	5	0.0	8	0.0	11	0.0
2	0.0	5	8.5	8	0.0	11	0.0
2	7.1	5	0.0	8	35.3	11	0.0
2	47	5	0.0	8	16.7	11	0.0
2	0.0	5	19	8	33.8	11	0.0
2	17.8	5	22.5	8	8.1	11	0.0
2	0.0	5	51.8	8	46	11	0.0
2	8.1			8	56.3		
2	0.0	6	35.5	8	36.3		
		6	44.1				
3	0.0	6	0.0	9	11.3		
3	74.2	6	25.6	9	0.0		
3	0.0	6	34.7	9	7.2		
3	21.6	6	0.0	9	20.3		
3	25	6	80.8	9	26		
3	37.8	6	65.2	9	16.8		
3	33	6	48.5	9	0.0		
3	24.9	6	54.7	9	44.8		
3	0.0	6	28.9	9	39.4		
3	78.5	6	81.4	9	41		
3	29	6	0.0	9	0.0		
3	48.1			9	33.7		
3	13.8			9	41.4		
3	43.6			9	35.7		
3	50.6						

10 MG/ML
PERCENT MUCOSAL LESION - INDIVIDUAL EXPLANTS

TEST ARTICLE	PERCENT LESION	TEST ARTICLE	PERCENT LESION	TEST ARTICLE	PERCENT LESION
1	0.0	4	0.0	7	0.0
1	0.0	4	54.6	7	0.0
1	22.6	4	29.5	7	31.9
1	0.0	4	18.3	7	0.0
1	3.5	4	0.0	7	23.4
1	10.9	4	33.6	7	0.0
1	23.6	4	29.6	7	21.5
1	10.5	4	14.3	7	0.0
1	6.1	4	15.5	7	5.3
1	0.0	4	24.1	7	0.0
1	0.0	4	30.3	7	17.9
1	17.4	4	37.2	7	23.5
1	0.0	4	12.5	7	0.0
1	14.6	4	9.5	7	21.3
1	41.5	4	32.8	7	0.0
2	54.8	5	0.0	8	0.0
2	37.1	5	0.0	8	0.0
2	66.7	5	0.0	8	27.8
2	0.0	5	50.7	8	0.0
2	8.2	5	44.2	8	0.0
2	0.0	5	10.3	8	31.8
2	48.8	5	33.7	8	13
2	0.0	5	0.0	8	33.1
2	0.0	5	56.8	8	27.8
2	46.9	5	25	8	0.0
2	50.3	5	31.2	8	32
2	6.0	5	0.0	8	0.0
2	54.6	5	30.8	8	0.0
2	68.1			8	3.7
		6	37.5	8	0.0
3	41.2	6	15.1		
3	14.7	6	17.5	9	10.1
3	0.0	6	19.3	9	0.0
3	30.4	6	0.0	9	0.0
3	15.8	6	0.0	9	0.0
3	63.8	6	14.3	9	30
3	34.7	6	13.8	9	0.0
3	15	6	6.3	9	0.0
3	53.7	6	12.9	9	0.0
3	30	6	9.9	9	36.2
3	74.9	6	27.4	9	18.3
3	50.1	6	51.2	9	0.0
3	15.5	6	0.0	9	40.6
3	6.8	6	55	9	0.0
				9	0.0
				9	28.1

STATISTICAL SUMMARY OF HISTOMORPHOMETRIC ANALYSIS
OF MICROSCOPIC LESIONS AT 100 UG ML LEVEL

** MATERIAL	MEAN	N	STANDARD DEVIATION	* SIGNIFICANCE NONPARAMETRIC
1	10.05	15	12.16	3,10
2	7.14	14	9.49	3,10,11
3	27.58	15	26.16	1,2,4,7,8
4	0.00	8	0.00	3,6,10,11
6	16.88	13	14.82	4,10
7	12.86	15	18.62	3,10
8	11.09	14	15.47	3,10
9	13.71	13	12.44	10
10	35.12	13	27.17	1,2,4,6,7, 8,9
11	23.56	14	25.58	2,4

* SIGNIFICANCE DETERMINED VIA NON-PARAMETRIC ANOVA AT THE 0.05 LEVEL
** KEY TO MATERIALS
1=MEDIA CONTROL, 2=GLASS BEADS, 3=ASBESTOS, 4=IRON WHISKERS, 6=AL-DUST,
7=KS-2 GRAPHITE, 8=MICRO-260 GRAPHITE 9=PRINTEX L CARBON BLACK,
10=NI GRAPHITE, 11=BRASS DUST

STATISTICAL SUMMARY OF HISTOMORPHOMETRIC ANALYSIS
OF MICROSCOPIC LESIONS AT 1 MG/ML LEVEL

** MATERIAL	MEAN	N	STANDARD DEVIATION	* SIGNIFICANCE NONPARAMETRIC
1	10.05	15	12.16	3,4,6,7
2	11.07	15	14.72	3,4,6
3	32.01	15	24.40	1,2,10,11
4	30.42	14	21.02	1,2,11
5	24.21	14	25.68	11
6	38.42	13	28.08	1,2,8,10,11
7	25.76	15	27.78	1,11
8	21.00	15	19.08	6,11
9	22.69	14	16.89	11
10	14.91	14	25.31	3,6
11	0.00	14	0.00	3,4,5,6,7,8,9

* SIGNIFICANCE DETERMINED VIA NON-PARAMETRIC ANOVA AT THE 0.05 LEVEL
** KEY TO MATERIALS
1=MEDIA CONTROL, 2=GLASS BEADS, 3=ASBESTOS, 4=IRON WHISKERS, 5=GRAPHITE FIBERS, 6=AL-DUST, 7=KS-2 GRAPHITE, 8=MICRO-260 GRAPHITE 9=PRINTEX L CARBON BLACK, 10=NI GRAPHITE, 11=BRASS DUST

STATISTICAL SUMMARY OF HISTOMORPHOMETRIC ANALYSIS
OF MICROSCOPIC LESIONS AT 10 MG/ML LEVEL

** MATERIAL	MEAN	N	STANDARD DEVIATION	* SIGNIFICANCE NONPARAMETRIC
1	10.05	15	12.16	2,3
2	31.54	14	27.37	1,7,8,9
3	31.90	14	22.41	1,6,7,8,9
4	22.79	15	14.77	7
5	21.75	13	21.20	
6	18.68	15	17.27	3
7	9.65	15	11.90	2,3,4
8	11.28	15	14.52	2,3
9	10.89	15	15.37	2,3

* SIGNIFICANCE DETERMINED VIA NON-PARAMETRIC ANOVA AT THE 0.05 LEVEL
** KEY TO MATERIALS
1=MEDIA CONTROL, 2=GLASS BEADS, 3=ASBESTOS, 4=IRON WHISKERS, 5=GRAPHITE FIBERS, 6=AL-DUST, 7=KS-2 GRAPHITE, 8=MICRO-260 GRAPHITE 9=PRINTEX L CARBON BLACK

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APPENDIX D

STUDY PROTOCOL

TECHNICAL PROTOCOL FOR IN VITRO TOXICITY ASSESSMENT OF PARTICULATES IN TRACHEAL ORGAN CULTURE

Study Number: G6695-0400

Sponsor: Chemical Research and Development Center

Sponsor's Toxicologist: Sandra Thomson, Ph.D.

Test Facility: Battelle Columbus Laboratories
505 King Avenue
Columbus, Ohio 43021

Study Director
and
Principal Investigator: Michael E. Placke, Ph.D.

Test Substances: There will be 8 metal particulates (Graphite fibers, polycrystalline iron wiskers, aluminum dust, nickel-coated graphite fibers, brass dust, Printer L, micro 260 synthetic graphite fibers, and K-2 natural graphite fibers) examined in this study. Characterization of these materials as to purity and stability will be the responsibility of the sponsor.

Records: All records that would be required to reconstruct the study and to demonstrate adherence to the protocol. The stipulations of this protocol are to be implemented in conformance with the Good Laboratory Practice Regulations (40 CFR, Part 172, EPA) for nonclinical studies. However, this study is not intended for submission to any regulatory agency and will not be listed on Battelle's Master Schedule.

I. Objective: The purpose of this study is to assess the in vitro acute and subacute toxic effects of the sponsor's test articles on the respiratory epithelium of hamster tracheal organ cultures and based upon comparisons with historical control substances, provide a rank order of the relative toxicity of each test article. Toxicity will be assessed based on microanatomical and histomorphometric changes.

II. Rationale: The tracheal organ culture model has been shown to be an effective short-term in vitro assay for the detection of cytotoxic and genotoxic

damage induced by select particulate compounds. The hamster was chosen as a donor animal based on the large volume of data available in the scientific literature on hamster tracheal explants exposed to a variety of xenobiotics.

III. Experimental Design: The project will be divided into 2 separate studies. The first will be a range-finding study to determine the toxic potential of each test article, the type(s) of lesion(s) each may produce and to identify the concentration at which extensive cytotoxic changes are most likely to occur. The second phase will be a definitive study to detail and quantify the types of lesions produced following in vitro exposure to the test articles and estimate the relative toxic effects of each test compound. Toxic changes induced by the test materials will be compared to a known positive (crocidolite asbestos) and negative (glassbeads) control, in addition to untreated explants maintained only in tissue culture media.

A. Characterization of Test Articles: Prior to explant studies, each particulate will be characterized as to its general physical characteristics mass medium diameter, and its properties while suspended in aqueous tissue media. This data will be used to calculate relative and effective doses of each compound and to assure individual particles (not conglomerates) of each test article are available for cellular interaction during exposure periods. In addition, the concentration of silica (SiO_2) within each test and control particulate will be determined by emission or colorimetric spectrophotometry methods. (Actual method employed will be based on relative concentration of silica in each sample).

B. Tracheal Organ Cultures: Sufficient numbers of five to six week old female, golden Syrian hamsters to provide the required number of organ explants will be obtained from Charles Rivers Laboratory. Upon receipt each animal will be examined and its general health assessed. Sera will be collected from 5 male and 5 female animals for serological evaluation. Serum samples will be sent to Microbiological Associates; Bethesda, MD and will be tested for titers to Sendai, Pneumonia Virus of mice, RCV/SDA, Kilham Rat Virus and Mycoplasma pulmonis. Tissues will be extracted from the hamsters within 2-14 days receipt. Animals will be anesthetized with sodium pentobarbital, the anterior, ventral one-half of their body clipped free

of hair and disinfected, and the trachea aseptically removed and placed in petri dishes containing phosphate buffered saline (PBS) with 1% penicillin, streptomycin and fungizone (PSF). Semi-circular tracheal rings, measuring approximately 2-4 mm^2 will be plated serosal side down onto 35 mm, 6- well culture dishes (5 explants/well) and media added. Pooled explants from several animals (approximately 3-4 hamsters) will be distributed randomly to each of the 6 wells (a total of 30 explants per dish. The media shall be minimum essential media (MEM-78-5048, Gibco Laboratories) supplemented with insulin, hydrocortisone hemiacetate, retinyl acetate, antibiotics and antimycotics. Media will be changed every other day. Explants will be incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Explants will be permitted to acclimate to culture conditions for 1-2 days prior to exposure.

C. Explant Exposure: The test and control particulates will be suspended at selected concentrations in culture media, vortexed and sonicated to assure a homogeneous suspension of single particles. The suspensions shall be prepared on a weight/volume basis. Each test article will be carefully weighed on an analytical balance and suspended in the appropriate amount of measured media. Analytical analysis of the formulation will not be conducted. The particulate-containing media will be pipetted onto the muscosal surface of the tracheal explants and the tissues incubated for 2 hours. After the exposure period, the media will be removed and fresh media added. One half of the explants will be collected at 1 week and the other one half of the explants collected 3 weeks following exposure.

D. Dose Regimen: The range finding study will be conducted as shown below:

Group	Test Substance	No. Wells/No. Explants	Concentration
1 a&b	x	4/20	1 µg/ml
2 a&b	x	4/20	10 µg/ml
3 a&b	x	4/20	100 µg/ml
4 a&b	x	4/20	1 mg/ml
5 a&b	x	4/20	10 mg/ml
6 a&b	x	4/20	100 mg/ml

Group "a" explants (2 wells total of 10 explants) will be collected at 1 week following exposure, group "b" explants (2 wells total of 10 explants) will be collected 3 weeks following exposure. The above design will be repeated for each of the 8 test articles, asbestos and glass beads. Four wells of explants (20 explants) exposed to media alone will be incubated at each time point as untreated controls.

The definitive study will be conducted in a similar fashion, incorporating 3 concentrations per test article (determined by results from the range finding study). A protocol amendment approved by the study director and project monitor will specify the concentrations of each test and control material to be used in the definitive study. There will be 6 wells of 5 explants each (30 explants) exposed for 2 hrs. to each concentration of test or control article. Three wells (15 explants) will be collected 1 week after exposure while the remaining explants will be collected 3 weeks post-exposure. Six wells (30 total explants) exposed to media alone will be included at each collection as untreated controls.

IV. Histopathology:

Explants will be collected in 10% neutral buffered formalin, paraffin embedded 5 per block and 3-5 um thick cross-sections will be taken approximately 100-150 um from the tissue face. These will be stained with hematoxylin and eosin and evaluated by light microscopy. The type, incidence and severity of mucosal lesions identified in tissues from the range-finding study will be summarized according to compound and dose for each collection time. Doses for the definitive study will be selected based upon results and observations made from the range-finding study. Exposure levels will be selected in an attempt to avoid extensive acute toxicity (mucosal degeneration and necrosis) and to provide a dose response development of proliferative or metaplastic changes (if such lesions were identified in the range-finding study). If no proliferative/metaplastic lesions are identified in the preliminary study, then the maximum tolerated dose (MTD), defined as the highest concentration that does not cause greater than 50% mucosal necrosis, will be the high dose used, with 2 additional doses at log intervals below the MTD selected for the definitive study. Duplicate tissues sections, separated by 300 um will be made of each block of explants collected three weeks post-exposure from the definitive study. These latter sections will be used for both qualitative assessment and histomorphometric analysis.

Morphometric Analysis: All explants collected at 3 weeks post-exposure in the definitive study will be morphometrically analyzed in order to quantitate proliferative and/or metaplastic lesions. The microscopic image of each duplicate section will be digitized onto a computer assisted image analysis system and the total crosssectional area of each tracheal explant mucosa determined. The total area within the mucosa containing hyperplastic, dysplastic, anaplastic, or metaplastic changes will then be determined. Final results will be expressed as the percent mucosal area of alterated epithelium (as defined above). Duplicate values for each explant (serial sections) will be averaged. Group means and standard deviations will be determined and statistically analyzed according to the methods detailed in Appendix I.

VI. Data and Tissue Retention: All raw data and data summaries will be retained by Battelle and held in Battelle's archive facility. Copies of the raw data and summaries will be sent to the Sponsor. All microscopic slides, paraffin blocks, and wet tissues from this study will be retained by Battelle until six months after submission of the final report. At the end of six months all slides blocks and wet tissues will be returned to the Sponsor or his designated archival facility.

VII. Interim Report: A draft interim report will be submitted within 5 weeks of the final collection of tissues from the range-finding study. The report will include the type, incidence and severity of all mucosal lesions according to compound, dose and time, with a recommendation for doses to be used in the definitive study.

VIII. Final Report: A draft final report will be submitted within 7 weeks of the final collection of tissues from the definitive study. The report shall include summary data from the range-finding study, the type, incidence and severity of mucosal lesions observed in the explants, and morphometric data of all explants collected at 3 weeks from the definitive study.

XI. Protocol Changes: If, after the study is underway, it be[illegible] necessary to change the approved protocol, verbal agreement to make this change should be made between the Study Director and Sponsor's project monitor. As soon as is practicle, this change and the reasons for it should be stated in writing for signature and inclusion in the study file.

Estimated Starting Date: January 20, 1986

Submission of Interim Report: March 24, 1986

Submission of Draft Final Report: June 23, 1986

Approval:

Battelle — Michael E. Placke — 12-23-85
Michael E. Placke, Ph.D.
Study Director — Date

C.R.D.C. — Sandra Thomson — 6 Jan 86
Sandra Thomson, Ph.D.
Project Monitor — Date

Reviewed by: Richard A. Stanko for — 12-23-85
Ramona Mayer
Quality Assurance Officer
Battelle Columbus Laboratories — Date

The statistical evaluation will be made by analysis of variance techniques. Provided that Bartlett's test of homogeneity of variance is not significant, treated groups will be compared to the control group using a one-way analysis of variance and Dunnett's t-test. If Bartlett's test is significant, comparisons with the control group will be made by a t-test technique which makes allowance for unequal variance. In this latter case, Wilcoxon's rank sum test will also be applied. Regression analysis on test group levels should be performed. All statistical tests will be conducted at a 5 percent, two-sided risk level.

NOTE: Suitable reference for these techniques are Snedecor, G. W. and Cochran, W. G., Statistical Methods, 6th Edition, Iowa State Univ. Press (1967).

Analysis of variance	pp. 270-277
Bartlett's test	pp. 296-298
Wilcoxon's rank sum test	pp. 130-132
t-test, unequal variances	pp. 114-116

PROTOCOL AMENDMENT 1

Technical Protocol for In Vitro Toxicity Assessment of Particulates in Tracheal Organ Culture.

Amendment: Section III. Experimental Design Part D. Dose REgimen.

The concentrations of test article for explant exposure to be used for the definitive study will be as follows:

DOSE LEVELS FOR DEFINITIVE STUDY

Glass beads	10 mg/ml	1 mg/ml	100 µg/ml	
Asbestos	10 mg/ml	1 mg/ml	100 µg/ml	
Iron Wiskers	10 mg/ml	1 mg/ml	100 µg/ml	
Graphite Fibers	10 mg/ml	1 mg/ml	100 µg/ml	
Aluminum Dust	10 mg/ml	1 mg/ml	100 µg/ml	
K-2 Graphite	10 mg/ml	1 mg/ml	100 µg/ml	
Micro 260	10 mg/ml	1 mg/ml	100 µg/ml	
Printers L	10 mg/ml	1 mg/ml	100 µg/ml	
Ni-coated Graphite	1 mg/ml	100 µg/ml	10 µg/ml	
Brass Dust	1 mg/ml	100 µg/ml	10 µg/ml	1 µg/ml

Reason: The doses were selected based upon results of the range-finding study.

APPROVED:

Michael E. Placke 4-28-86

Michael E. Placke, Ph.D. Date
Study Director

Sandra Thomson 5/12/86

Sandra Thomson, Ph.D. Date
Sponsor Representative

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